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PHYSIOLOGICAL REVIEWS

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PHYSIOLOGICAL REVIEWS

VOL. 25 JULY, 1945 No. 3

THE RELATION OF ADRENALINE TO ACETYLCHOLINE IN THE NERVOUS SYSTEM

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Most people think of adrenaline and acetylcholine as antagonists. The one raises the blood pressure, the other lowers it; the one inhibits intestinal movement, the other increases it; the one dilates the pupil, the other constricts it. Adrenaline produces the effects of sympathetic stimulation, and acetylcholine those of parasympathetic stimulation, and since the sympathetic and parasympathetic systems produce effects almost always in opposite directions, there is abundant reason for the view that adrenaline and acetylcholine have antagonistic actions.

It is now known that not only are parasympathetic effects imitated by acetylcholine, but that acetylcholine is released when an impulse is transmitted from the vagus endings to cardiac tissue (for example), and the transmission of the impulse is due to the acetylcholine. Acetylcholine is also liberated when an impulse is transmitted from somatic motor nerve endings to skeletal muscle, and when an impulse passes from a preganglionic fibre of the sympathetic system to a post-ganglionic fibre. Many believe that at the neuromuscular junction in skeletal muscle and across the sympathetic ganglion, the transmission of the impulse is effected by acetylcholine there too. Finally some evidence has been obtained that acetylcholine is released when impulses cross the synapses of the spinal cord, so that there also, at some synapses, acetylcholine may be the transmitter.

What part, if any, does adrenaline play at these points of transmission of a nervous impulse at which acetylcholine is liberated? Is the transmission of a nervous impulse affected by the presence of adrenaline? Is the effect of acetylcholine, deliberately applied at these points, modified by the presence of adrenaline? These are the main questions now to be discussed.

Adrenaline action on denervated muscle. The earliest observations which suggested that adrenaline modifies the action of acetylcholine were made on denervated skeletal muscle. It has long been known that when the postganglionic sympathetic fibres to the pupil of the eye degenerate as a result of extirpating the superior cervical ganglion, the pupil becomes much more sensitive to the action of adrenaline. In the same way, when the motor fibres to skeletal muscle degenerate, the muscle becomes much more sensitive to the action of acetylcholine and of drugs having a similar action, such as nicotine. This increase in sensitivity is very great, and indeed the response of denervated muscle to acetylcholine was known long before its action on normally innervated muscle was discovered, since special methods are needed to display this. Philippeaux and Vulpian (1) showed that when the hypoglossal nerve had been allowed to degen-

erate, stimulation of the chorda-lingual nerve, or of the chorda tympani alone, caused a contraction of the denervated tongue. Heidenhain (2)showed that this denervated tongue also behaved differently from innervated tongue in that it contracted when an intravenous injection of nicotine was given. Frank, Nothmann and Hirsch-Kauffmann (3) found that acetylcholine not only acted like nicotine, but did so in very small doses. We see, then, the explanation of the observation of Philippeaux and Vulpian: stimulation of the chorda tympani liberates acetylcholine, and the tongue, sensitized by denervation, contracts in response to it. Other phenomena have received a similar explanation. Sherrington (4) observed that the muscles of the cat's leg and foot when denervated by section of the spinal nerve roots, contracted when the cut end of the sciatic nerve was stimulated six weeks later. The work of Hinsey and Cutting (5) and of Bülbring and Burn (6) showed that the fibres responsible for the effect were the sympathetic fibres which remained unaffected by the root section, and the last-named authors (7) showed that some of the sympathetic fibres supplying blood vessels in the muscles liberated at their terminations, not adrenaline (or sympathin), but acetylcholine. Here again the explanation of the Sherrington phenomenon is that stimulation of the sympathetic chain liberates acetylcholine at endings in the muscle blood vessels; the muscles of the leg and foot, sensitized by denervation, contract in response to it. (Those wishing to read more of the pharmacology of denervated muscle should consult the paper by Dale and Gasser (8), in which they examined the different substances known to cause contraction, and observed that it was the nicotine-like property of acetylcholine and other quaternary bases which enabled them to act in this way.)

The effect of adrenaline on contractions, or contractures, caused by drugs in denervated muscle has been tested by several workers, but the chief contribution to the evidence was made by Dale and Gaddum (9). They found that if the contraction of the denervated muscle was induced by injection of acetylcholine either intravenously or intra-arterially, then the effect of injecting adrenaline beforehand depended on how long beforehand the injection was made. If the acetylcholine was injected immediately after the injection of adrenaline, then acetylcholine usually failed to produce a contraction at all; on the other hand when the interval between the injections was longer, about one minute, then the effect of the acetylcholine was increased, the contracture being stronger and more persistent. Thus after the injection of adrenaline, there were two phases of the acetylcholine response, an early phase of depression and a later phase of augmentation. The two phases were nearly always observed in the denervated gastrocnemius of the cat, though sometimes the second phase of augmentation was not seen. In the denervated tongue on the other hand the phase of depression was only once seen, and the usual effect was augmentation and, especially, prolongation. Similar results have since been obtained by Luco (10).

Dale and Gaddum then carried out a simple experiment on an isolated strip of denervated diaphragm. It seemed likely that the double effect of adrenaline

might be due to its vascular action complicating another action. To avoid this complication they looked for a skeletal muscle which might be expected to survive for some hours without a circulation provided it was suspended in a well-oxygenated bath of Ringer's solution. They chose the diaphragm of a young cat. One-half of the diaphragm was denervated by removal of a portion of one phrenic nerve, and after 14 days' interval, a strip of the denervated side of the diaphragm was suspended in a bath at 37°C. This strip contracted when 2 microg. of acetylcholine was added (to make a concentration of 1 in 7.5 millions). After washing out, the strip relaxed, and it was then observed that if the same dose of acetylcholine was added in the presence of 0.2 mg. adrenaline put in the bath 6 minutes before, then the contraction was greater. The bath was again washed out. The acetylcholine contraction became still further augmented when a third dose was added, showing that the effect of the adrenaline persisted for some time after it was removed from the bath; only after two or three washings did this effect disappear. It is important to note that the contraction caused by tetramethylammonium iodide was also augmented by adrenaline as well as that caused by acetylcholine.

This very simple experiment illustrated what was then a quite new and is still a little known aspect of the relation of adrenaline to acetylcholine, in which these substances no longer appear as antagonists but rather as allies, the one potentiating the action of the other. Adrenaline itself is not known to have any action on normal skeletal muscle whether this be frog or mammalian muscle, but there are two observations which have been made concerning its action in denervated mammalian muscle. v. Euler and Gaddum (11) observed that adrenaline produced a slow contracture of the denervated canine muscle which runs along the upper lip of the dog. Similarly Bülbring and Burn (6) found that adrenaline produced a slow contracture of the denervated gastrocnemius in both the cat and the dog. The potentiation by adrenaline of the action of acetylcholine is not, however, peculiar to denervated muscle. Bülbring (unpublished observations) has observed that the contraction of the frog's rectus abdominis muscle, suspended in a bath, in response to acetylcholine of concentration 10^{-7} is potentiated by the addition to the bath of adrenaline in a concentration of 10^{-6}. Thus, we see that in skeletal muscle adrenaline potentiates the action of acetylcholine, the action of a substance which is now believed, since the work of Dale, Feldberg and Vogt (12), to be responsible for the transmission of the impulse from the motor nerve ending to the muscle itself.

The fact at once recalls the observations which have been made on the effect of adrenaline in augmenting the contraction of muscle partially fatigued. The earliest observations were made by Gruber (13) who showed that when a rapid series of interrupted tetani were applied to the sciatic nerve of the cat, and when the contractions diminished due to fatigue, the intravenous injection of adrenaline augmented the contractions. Likewise the observations of Orbeli (14) are recalled who showed that stimulation of the motor roots in the frog led to diminishing contractions which were augmented when the sympathetic fibres were stimulated in addition. We know that the sympathetic fibres liberate adrenaline

(or sympathin) and the augmentation described by Orbeli may represent the potentiation by this adrenaline of the acetylcholine liberated at the motor nerve ending. Gruber's observations have been confirmed by many subsequent workers, who have been divided in their opinion whether the action of the adrenaline was exerted at the neuromuscular junction or on the muscle itself. Bülbring and Burn (15) showed that much the greater part of the effect was due to an action at the neuromuscular junction, and so quite possibly related to the release of acetylcholine there.

Observations in the spinal cord. Between 1930 and 1941 little attention was paid to the observation made by Dale and Gaddum on the strip of cat's denervated diaphragm; it remained as a single observation in a paper full of other observations, and its possible connection with Gruber's work and with the Orbeli phenomenon was not pointed out. In 1941 Bülbring and Burn published observations concerning the transmission of impulses in the spinal cord (16). They made these observations on the lower part of the dog's spinal cord which was perfused with defibrinated blood. They elicited the flexor reflex and the knee jerk in one hind leg, which was perfused by a separate circulation of blood. Their experiments were directed to obtaining evidence that acetylcholine was responsible for the transmission of impulses across the synapses in the cord. Part of the evidence that acetylcholine is a transmitter at the neuromuscular junction is that acetylcholine given in a very small dose by intra-arterial injection at the point where the artery enters the muscle causes a typical muscle contraction (Brown, Dale and Feldberg, 17). In the same way, part of the evidence that acetylcholine may be the transmitter in the sympathetic ganglion is that acetylcholine injected into the perfused superior cervical ganglion causes a discharge along the postganglionic fibres (Feldberg and Gaddum, 18). Bülbring and Burn therefore attempted to demonstrate that when acetylcholine was injected into the blood going to the perfused spinal cord, a discharge of motor impulses from the anterior horn cells occurred, leading to contraction of the anterior tibial muscle in the separately perfused hind-leg. They were at first unable to demonstrate this; they discovered, however, that if adrenaline was present in the blood perfusing the spinal cord, the demonstration was quite easy, and when the acetylcholine was protected on its way through the vessels to the cord by a small dose of eserine given one minute earlier, then 1 microg. acetylcholine produced a series of contractions in which tensions more than 1.0 kgm. were developed. The point of interest was the need for the presence of adrenaline in order to demonstrate this action of acetylcholine.

Two other observations were also made. The contraction of the anterior tibial muscle in response to stimulation of the posterior tibial nerve (the flexor reflex) was small unless adrenaline was added to the blood perfusing the spinal cord. Here the normal transmission of an impulse from the sensory to the motor side of a reflex arc was so improved by adrenaline that the tension developed in the contracting muscle rose from 0.3 kgm. to 1.3 kgm. The other observation concerned the action of prostigmine. Prostigmine was found to have little or no effect on the flexor reflex. This was a surprising result since when

eserine was injected into the blood going to the spinal cord, the tension developed in the flexor reflex was often doubled. This result conformed with the earlier observations of Merlis and Lawson (19) on the effect of eserine on the flexor reflex. It had been expected that prostigmine would act in the same way. After the addition of adrenaline to the blood, however, the injection of prostigmine did increase the flexor reflex.

In this work on the spinal cord there was evidence that adrenaline augmented both the action of acetylcholine, and also that of prostigmine; since prostigmine by its anticholinesterase action increases the amount of acetylcholine it seemed possible that the synergism between adrenaline and prostigmine was essentially a synergism between adrenaline and the protected acetylcholine, but this will be discussed later.

Prostigmine in skeletal muscle. The relation found to exist between prostigmine and adrenaline in the spinal cord suggested that a similar relation might exist in skeletal muscle. Bülbring and Burn (20) therefore made experiments in which they recorded the tension developed in the gastrocnemius of the cat when the maximal single shocks were applied to the sciatic nerve every 15 sec. They injected prostigmine into the common iliac artery, and observed that after a dose of 0.02 mgm. a moderate increase in the tension developed at each contraction. When an injection of 0.02 mgm. adrenaline was given four minutes after the injection of prostigmine a further increase of the tension was recorded, about as great as that caused by the prostigmine. In other experiments they injected prostigmine and adrenaline together, comparing the effect with that of prostigmine alone, and found that the combination produced more than double the effect. The action of eserine was also increased by combining it with adrenaline, but the increase was slight; it was very much less than the increase in the effect of prostigmine.

The sciatic nerve was also stimulated at more rapid rates. At these more rapid rates prostigmine caused a greater rise in the tension, presumably because acetylcholine accumulated more rapidly; when adrenaline was then injected, the injection no longer caused a further increase, but now depressed the tension for a period. This depressant action was directly connected with the more rapid rate of stimulation for it disappeared when the initial slow rate was resumed. At fast rates of stimulation prostigmine in large dose itself caused depression, and adrenaline intensified this depression. These experiments so far indicated that adrenaline potentiated the effect of prostigmine, converting a small effect into a greater effect, transforming a maximal effect into the effect of excess, namely, a depression, and converting a depression into a still greater depression.

That excess of acetylcholine should depress the effect of nerve stimulation is easy to understand; when there is excess the number of enzyme molecules is insufficient to destroy the molecules of acetylcholine present, and many of these remain attached to the receptors on the cell, blocking the access of new molecules. If the size of the muscle response depends on the proportion of acetylcholine molecules released by the nervous impulse which are able to make effective connection with cell receptors, then the response will be depressed if some

receptors are blocked by undestroyed acetylcholine. To explain how adrenaline can potentiate the action of acetylcholine, so that the effect of a small dose is increased, while the effect of a larger dose is decreased, is not so easy. We can suppose that adrenaline increases the permeability of some membrane situated between the point of liberation of acetylcholine and the point of union with the cell receptors. By increasing the permeability, adrenaline increases the proportion of acetylcholine molecules which penetrate the membrane. If the amount of acetylcholine is small, this increase will result in a greater response; if however the amount of acetylcholine is large, the increase will result in a smaller response because there will be an excess of acetylcholine at the point of union with cell receptors.

Relation of adrenaline to prostigmine. The simplest view of this action of adrenaline is that it is basically the same as the action described by Dale and Gaddum on the denervated muscle of the cat's diaphragm. On the cat's diaphragm in a bath of Ringer's solution, adrenaline potentiates acetylcholine, so that it causes a larger contraction. When prostigmine is injected into the cat, and the sciatic nerve is stimulated, acetylcholine accumulates at the motor end plates; adrenaline injected while the accumulation persists potentiates this acetylcholine and causes a greater contraction.

Against this simple view there are certain arguments to be put forward. If the view were correct, adrenaline should potentiate the action of eserine as effectively as it potentiates the action of prostigmine. There is no doubt at all that it does not do so, either in skeletal muscle or in the spinal cord. That is to say, there is a special relation between adrenaline and prostigmine independent of the action of the latter in preserving acetylcholine from destruction by cholinesterase. Bülbring and Burn (20) obtained the clearest evidence of this on blood vessels. When the vessels of the hind leg of a dog were perfused with defibrinated blood by a pump, the injection into the artery cannula of 4 microg. of adrenaline caused the pressure in the cannula to rise and the venous outflow to be reduced. The injection of twice this dose produced a proportionally greater effect. Prostigmine was then injected into the artery cannula in a dose of 20 microg. This injection itself was followed by a moderate but prolonged rise in the arterial resistance, and in addition the action of the doses of adrenaline was increased so that their effect was doubled. After prostigmine, 4 microg. of adrenaline had the same effect as 8 microg. injected before. Thus the observation of Mendez and Ravdin (21) that prostigmine possesses some peripheral vasoconstrictor action was confirmed and it was also found that prostigmine affects the action of adrenaline on the blood vessels in much the same way as cocaine.

There is an additional reason for supposing that the potentiation of the effect of prostigmine on skeletal muscle by adrenaline is not simply a potentiation of the acetylcholine protected by prostigmine. When the gastrocnemius muscle of the cat is stimulated by an interrupted tetanising current applied to the sciatic nerve 120 times a minute, fatigue ensues, and the tension developed by each short tetanus slowly declines during 2 to 3 minutes and then is maintained

almost steady. As was first shown by Gruber (13) the injection of adrenaline at this point increases the tension developed, the increase rising to a maximum and then passing off. If, instead of injecting adrenaline, prostigmine is injected the tension at once falls, as was demonstrated by Briscoe (22). It should be noted that this fall occurs not only in fatigued muscle, but also in rapidly stimulated muscle which is not fatigued. Thus in muscle subjected to rapid stimulation so that fatigue is produced, adrenaline and prostigmine have opposite effects. The former increases the tension while the latter decreases it. This is a reversal of the position in muscle stimulated at a moderate rate in which prostigmine causes a large increase in tension, and adrenaline then causes a depression. The contrast was found to be still more striking when Bülbring and Burn injected prostigmine into rapidly-stimulated fatigued muscle, observing that it caused a decline of the tension, and then injected adrenaline, to find to their surprise that the adrenaline still caused a rise of tension. They had assumed that since prostigmine diminished the tension, an excess of acetylcholine must already be present, and expected that the "potentiation" of an excess, if it altered the tension at all, would depress it still more. If the dose of prostigmine is large and allowed to act for some time, the injection of adrenaline following prostigmine does in fact cause further depression beyond that produced by prostigmine, but the earlier stage remains to be explained.

The conclusion is difficult to avoid that there may be three actions of adrenaline. First the action by which the effect of acetylcholine on muscle is intensified; second the action by which the effect of prostigmine is intensified, and third an action now to be discussed. When Orbeli (14) first described how stimulation of the sympathetic rami increased the contraction of the gastrocnemius of the frog, fatigued by repeated stimulation of the motor roots, he explained the effect of sympathetic stimulation as restoring the power to transmit impulses to some of the motor end organs in which as a result of repeated stimulation transmission had failed. The idea has generally been expressed by saying that sympathetic stimulation, or adrenaline, "lowers the threshold" for the transmission of impulses at the neuromuscular junction. But in what does this lowering of threshold consist? Is it an action at the nerve ending at all? May it not just as well be an effect of sympathetic stimulation or of adrenaline on the transmission of impulses along the nerve?

Transmission along the course of the nerve. Bülbring and Burn (23) investigated the contractions of the gastrocnemius in response to maximal single shocks applied to the motor roots. The lower part of the dog's hindleg was perfused with defibrinated blood, and having prepared the lumbar sympathetic chain for stimulation, they were able to see the effect of the sympathetic on the motor root stimulation. The stimuli were applied at intervals of 10 sec. so that no question of fatigue at any point could arise. It was found that the stimulation of the motor roots became progressively ineffective, the tension developed in the gastrocnemius declining from 9 kgm. to zero. The tension could be promptly restored either by stimulation of the sympathetic chain for 2 minutes or by the addition of adrenaline to the perfusing blood. The restorative effect was at

first thought to be exerted at the neuromuscular junction, because there was no failure of the muscle to contract in response to direct stimulation, and no change in the tension developed by direct stimulation when the sympathetic was stimulated or adrenaline was added. A failure of transmission at the neuromuscular junction in the absence of fatigue had not been previously described, and it was then shown that at a time when stimulation of the motor roots became ineffective, stimuli applied to the sciatic nerve at a point near its entry into the muscle still produced a tension of nearly 6 kgm. That is to say, at a time when stimuli applied to the motor roots was ineffective, the same stimuli applied to the sciatic nerve near the muscle caused the gastrocnemius to contract. Transmission at the neuromuscular junction was still good but transmission along the nerve itself had become defective. It was further shown that application of single shocks to points on the sciatic nerve at increasing distances from the muscle produced contractions of decreasing tension. The injection of adrenaline into the blood, however, increased the distance from the muscle at which a response of given tension could be evoked. These experiments made it clear that sympathetic stimulation, or the presence of adrenaline, exerted a considerable effect on the transmission of impulses along the course of the motor nerves.

The addition of adrenaline to the perfusing blood, or the stimulation of the sympathetic chain of course produced vascular changes in the perfusion system; these were recorded as a rise in the arterial resistance to the inflow of blood and as a diminished venous outflow. It looked at first sight as though transmission along the motor fibres was improved when the blood flow was less. Changes in venous outflow and in arterial resistance, though indicating a total diminution in blood supply, give no indication of what may happen locally; thus it may be that adrenaline causes certain arteriovenous anastomoses to be shut with the result that capillary areas in the neighbourhood obtain a greater supply. It seems very probable that the improved transmission obtained by injecting adrenaline or by sympathetic stimulation was in fact due to vascular changes of the kind indicated rather than to any specific effect of adrenaline itself, since improved transmission along the motor fibres followed the addition to the perfusing blood of pituitary (posterior lobe) extract or of blood which had been standing, in which vasoconstrictor substances had accumulated. On the other hand against this explanation was the rate of recovery. When stimulation was applied to the sympathetic chain, within 30 sec. the tension developed in the gastrocnemius in response to single shocks applied to the motor roots had increased from 4 to 6 kgm., and within 60 sec. had risen to 9 kgm. Mere improvement in blood flow might be expected to take longer to exert so much effect. Moreover the effect on muscle tension persisted five or six times longer than the effect on blood flow. The fact remains that in the conditions of these experiments, adrenaline, whether injected or liberated at sympathetic nerve endings, was found to affect transmission along motor fibres in a striking manner.

Adrenaline on action potentials. That adrenaline affects the action potentials of nerve was later demonstrated by Bülbring and Whitteridge (24). They stimulated the sciatic nerve in the cat, having made a spinal, eviscerated and

adrenalectomised preparation, and recorded action potentials in the posterior tibial nerve. When submaximal stimuli were applied at a frequency of 1 per second, the injection of 5 microg. adrenaline caused an increase in the height of response of $\alpha\,\beta$ fibres which varied in nerves in good condition from 30 to 115 per cent. In experiments where the nerve had been incompletely guarded from fatigue, increases of 300 per cent in height of response were observed. It should be emphasized that this increase was observed with submaximal stimulation, and not when maximal stimuli were employed.

How is the effect of adrenaline on the transmission of impulses along the course of the sciatic nerve to be explained? Certainly the results of Bülbring and Whitteridge cannot be attributed to a mere alteration of blood supply. It is true that their best results were obtained in the spinal cat which often has a low blood pressure: but they were also obtained in the decerebrate cat or cat anaesthetised with chloralose, and both of these have a high blood pressure. The intravenous injection of 5 microg. adrenaline produces a concentration in the blood of a cat weighing 3 kgm. roughly equal to 0.3×10^{-7}, and this can have little vascular action.

Recently Nachmansohn has put forward the view that acetylcholine is concerned with the transmission of the nervous impulse along the nerve fibre as well as at the synapse. Fulton and Nachmansohn (25) argue that the main difficulty which has stood in the way of accepting the theory of humoral transmission at nerve endings has been that this theory has implied a basic difference in the method of transmission across a synapse and of transmission along a nerve. They point out that Gasser and Erlanger (26) came to the conclusion that conduction along fibres and across synapses is essentially the same process, and that the difference is only quantitative. If however it can be shown that transmission along a nerve involves the activity of acetylcholine, then this difficulty in the way of accepting the theory of humoral transmission disappears. Nachmansohn and Meyerhof (27) have found that in the electric organs of fishes there is a close parallelism between the voltage produced and the concentration of cholinesterase. This suggests a relation between acetylcholine and the electric discharge. More relevant to the present discussion, however, is their further finding of a high concentration of cholinesterase in the sheath of the giant nerve fibre of the squid, the concentration in the axoplasm being negligible. They suggest that the potential differences observed during nerve activity are closely connected with the metabolism of acetylcholine everywhere at or near the surface of the nerve cell (including the axon), and not only at the synapse. If it be true that transmission along nerve fibres involves the release of acetylcholine, then it is possible to relate the beneficial effect of adrenaline on transmission along the sciatic nerve to the other conditions in which adrenaline potentiates the action of acetylcholine. We see again this collaboration which seems to be a fundamental property of the two substances.

Motor paralysis by sympathetic stimulation. The parallel does not stop at simple potentiation of nerve transmission by adrenaline. Bülbring and Burn (23) found that while the ordinary effect of stimulating the sympathetic chain

was to augment the contraction of the dog's gastrocnemius when this was elicited by stimulation of the motor roots, in certain experiments in which a tetanus of 20 sec. duration was applied to the roots, stimulation of the sympathetic chain produced, during the stimulation, an abolition of the muscle response followed by augmentation of the muscle response only when the sympathetic stimulation had stopped. Here was seen a paralysis of motor response as a result of sympathetic stimulation. The picture called to mind was that of creatures being "paralysed by fear," so frightened that they are unable to move, being "rooted to the spot." The failure of the motor response in the experiment described must have been due to the liberation of adrenaline, and this when liberated acted in such a way that the transmission of the impulse along the nerve or at the nerve ending by the acetylcholine mechanism was interrupted. We can suppose that the effect was due to an interplay between adrenaline and acetylcholine similar to that observed by Dale and Gaddum (9) when sympathetic stimulation or adrenaline injection abolished the effect of an immediately preceding dose of acetylcholine in denervated muscle.

Summary of actions in relation to skeletal muscle. It was stated earlier that at least three actions of adrenaline in relation to skeletal muscle must be considered. First the action by which the action of acetylcholine on denervated mammalian or normal frog muscle is intensified; second, the action by which the effect of prostigmine is intensified; and third, the action on neuromuscular transmission which has now been shown to apply to conduction along the nerve fibre also. Orbeli certainly considered that the improvement in the effect of motor root stimulation due to sympathetic stimulation was exerted by lowering the threshold at the neuromuscular junction; the observations of Bülbring and Burn and of Bülbring and Whitteridge clearly concern transmission along the nerve, which adrenaline affects perhaps by modifying the threshold for excitation. It seems probable that it will soon be generally recognised that effects produced on transmission along the nerve cannot be distinguished from similar effects occurring at the neuromuscular junction.

The sympathetic ganglion. Gaskell, in his book on *The Involuntary Nervous System* (28), points out the close connection between the chromaffine cells which form adrenaline, and the cells of the sympathetic ganglia. "In the adult mammal the chromaffine system is confined to the medulla of the suprarenals, but in the embryo chromaffine cells are found in close connexion with sympathetic cells; a specially large mass known as Zuckerkandl's body which is found at the bifurcation of the abdominal aorta, lasts till birth closely connected with the inferior mesenteric ganglia. As we pass downwards in the animal kingdom, we find the chromaffine cells and sympathetic cells in close contact, even in the adult condition. In the Amphibia, where the sympathetic ganglia are arranged much in the same way as in the Mammalia, the chromaffine cells and the sympathetic cells are mixed close together in every ganglion" (pp. 139-140, loc.cit.). Recently the work of Stöhr (29) has again called attention to the presence of chromaffine tissue surrounding the ganglion cells of sympathetic ganglia.

The first work on humoral transmission in sympathetic ganglia was that of Kibjakow (30) who made a valuable contribution to physiological methods by describing the perfusion of the superior cervical ganglion of the cat with Locke's solution. The perfusion is carried out in a cat in which the normal circulation of the blood is maintained in the rest of the body. In the course of his work Kibjakow observed that if he collected the Locke's solution coming out of the ganglion during a period of prolonged stimulation, and then re-perfused this fluid through the ganglion, the threshold for stimulation was lowered. Using Kibjakow's method, Feldberg and Gaddum (18) demonstrated that stimulation of the preganglionic fibres caused the liberation of acetylcholine from the ganglion, and from then on attention was focussed on acetylcholine as the humoral transmitter.

The first evidence that adrenaline played a physiological rôle in the sympathetic ganglion was that of Marrazzi (31) who published evidence that adrenaline causes depression of the nervous impulse in its passage through the superior cervical ganglion. He made observations in cats and rabbits anaesthetized with pentobarbital, recording the action potentials in the postganglionic fibres when submaximal shocks at a rate of 2 per sec. were applied to the preganglionic trunk. He observed that, when adrenaline was injected intravenously in doses from 5 to 500 microg. the action potentials were reduced. Marrazzi did not observe any stage in which the action potentials were increased and the one effect he recorded was that of ganglionic depression. He also observed that the injection of ephedrine depressed the action potentials in the postganglionic fibres, but the doses he used were very large, being 10 mgm. per kgm. In order to exclude any vasoconstrictor action of adrenaline as the cause of the ganglionic effect, Marrazzi arrested the circulation by piercing the heart while still recording the action potentials. During the first five minutes after death there was no decrease, and indeed there was an increase in the height of the spike, which later became greater. It is interesting to note that when Marrazzi tested the effect of larger doses of adrenaline (0.25 mgm.) he observed that in recovery from the initial depression the spike height rose to a slightly higher level than the original.

In 1942 Bülbring and Burn (32) published evidence which indicated that adrenaline exerted two effects on ganglionic transmission, one an augmentor action, and the second a depressor action like that described by Marrazzi. It had been shown earlier, (Burn (33)), that the vasoconstriction produced in a perfused hindleg when the sympathetic chain was stimulated, was greater in the presence of adrenaline. This change was believed to be produced at the postganglionic terminations. The recent paper by Stöhr (29) describing the presence of chromaffine tissue in sympathetic ganglia indicated a different possibility, that adrenaline might improve transmission in the sympathetic ganglia. Bülbring and Burn therefore made experiments in a double perfusion system in the dog. They arranged one circuit of defibrinated blood to perfuse the ganglia of the lateral sympathetic chain from the level of the kidney downwards, and a second circuit to perfuse the corresponding hindleg. They were then

able to test the effect of different concentrations of adrenaline in the ganglion circuit on the effect of stimulating the sympathetic chain. The vasoconstrictor response in the vessels of the hindleg was recorded to measure this effect.

In the leg circuit a steady concentration of adrenaline was maintained throughout the observations. In the absence of any adrenaline from the ganglion circuit, a very small vasoconstrictor response was obtained in the leg vessels when the sympathetic chain was stimulated. When adrenaline was added to the venous reservoir of blood in the ganglion circuit at a rate of 4 microg. per min. (the total volume of blood in the circuit being 600 cc.), then stimulation of the preganglionic fibres gave a greatly increased response. It was concluded that the initial effect of adrenaline on the sympathetic ganglia was to improve the transmission.

A different effect of adrenaline was seen when the amount of it in the blood passing through the ganglia was raised further. When the rate of adding adrenaline to the venous reservoir increased from 4 microg. per min. to 10 microg. per min. the response to stimulating the preganglionic fibres diminished, indicating depression of the ganglionic transmission. This second result was in agreement with observations of Marrazzi.

The pressor action of acetylcholine. In the foregoing experiments the sympathetic ganglia received stimuli from the preganglionic fibres. It is however also possible to stimulate the ganglia by the injection of acetylcholine. To observe this, atropine must be given to exclude the vasodilator and cardio-inhibitory actions of acetylcholine, and the doses employed must be relatively large. Bülbring and Burn made observations in spinal cats and observed the pressor effect of injecting acetylcholine in doses of 0.4 to 0.6 mgm. at regular intervals. They found that when adrenaline in small amount, e.g., 5 microg., was interposed between two acetylcholine injections, the effect of the second injection was often (though not always) augmented.

When however a larger amount of adrenaline was used, 0.03 mgm., the effect of the second injection of acetylcholine was depressed, the depression slowly passing off in the course of 30 min. or longer. This depressant action of adrenaline was well shown in experiments on atropinised spinal cats when a slow intravenous infusion of adrenaline was given. The pressor effect of acetylcholine was almost abolished. When the adrenaline infusion was stopped, and the blood pressure, which then fell, was restored to the same height by an intravenous infusion of pituitary (posterior lobe) extract, the pressor effect of acetylcholine was fully displayed. These observations were made in cats from which the suprarenal glands were removed, so that the rise of blood pressure produced by acetylcholine was due to ganglionic stimulation alone without stimulation of the suprarenal medulla. The depressant action of adrenaline resulting in paralysis of the pressor effect of acetylcholine in the atropinised cat has also been observed by Stehle and Melville (34).

Effect of adrenaline on splanchnic stimulation. The experiments in which acetylcholine was used to stimulate ganglia showed evidence of both effects of adrenaline, the augmentation by small doses and the depression by larger ones.

It was however much easier to observe the depressant effect of the large doses of adrenaline. The reverse was true in experiments in which Bülbring and Burn stimulated both splanchnic nerves in spinal cats from which the suprarenal glands were removed. The stimulation was maximal, and was effected by condenser discharges at rates varying from 8 to 48 per sec. The result of stimulation was recorded as the rise of blood pressure in the carotid artery. Continuous intravenous infusion of adrenaline at rates varying from 4 to 15 microg. per min. increased the rise of pressure produced by a given stimulation by 70 to 110 per cent. No similar change was produced by continuous intravenous infusion of pituitary (posterior lobe) extract. The increase in the pressor response to splanchnic stimulation during an intravenous infusion of adrenaline might of course have been due to a change in the vessels; it was demonstrated however that there was no corresponding change in the pressor effect of adrenaline injected in a single dose. For example, in one experiment before the intravenous infusion of adrenaline, splanchnic stimulation had a pressor effect little more than half that of 7 microg. adrenaline; during adrenaline infusion (2.5 microg. per min.) the pressor effect of splanchnic stimulation was increased so as to be equivalent to that of 7 microg. The inference was that the increased pressor effect of splanchnic stimulation was not due to a peripheral effect on the vessels, but was consistent with improved ganglionic transmission.

In these experiments adrenaline was infused intravenously at rates as high as 17 microg. per min., but at no rate was the effect of splanchnic stimulation depressed. In contrast to intravenous infusion, a single large dose of adrenaline, e.g., from 0.04 mgm. to 0.3 mgm., diminished the pressor effect of splanchnic stimulation for periods varying according to the dose of adrenaline injected. The diminution lasted 30 min. after the injection of 0.08 mgm., more than 45 min. after the injection of 0.12 mgm., and over two hours after the injection of 0.3 mgm.

Results in the perfused ganglion. The observations so far described were made in circumstances in which it was difficult to be certain that effects following the application of adrenaline were exerted in the ganglion and at no other point. Bülbring (35) has therefore studied the action of adrenaline in the superior cervical ganglion of cat when perfused with Locke's solution by the method first described by Kibjakow (30). The preparation was made in cats with normal circulation except in the perfused ganglion, and since adrenaline was injected into the perfusion system, the effect of the adrenaline was sharply restricted to the ganglion. She observed that when a constant submaximal stimulus was applied at intervals of 2 to 3 min. to the preganglionic fibres, the injection of small doses of adrenaline into the perfusion fluid increased the response, which was measured as a contraction of the nictitating membrane. The doses employed were from 0.01 to 0.1 microg. The same increase was seen when adrenaline was added to the perfusion fluid in a concentration of 1 in 100 or 1 in 200 million. The increase was observed only when the stimulation was submaximal and applied at a rate less than 8 per sec. Larger doses of adrenaline

were found to have the opposite effect, and to cause depression of the response. The contractions in response to maximal stimulation were also depressed by these larger doses.

Striking results were obtained when the ganglion was stimulated by injections of acetylcholine. Doses of 10 microg. acetylcholine injected into the fluid perfusing the ganglion caused small but regular effects; when an injection of 0.05 microg. adrenaline was interposed, there was a very large increase in the effect of acetylcholine, which steadily augmented and then once more declined. Larger doses of adrenaline reduced the effect of acetylcholine. The injection of adrenaline had of course some effect on the rate of flow of the perfusion fluid through the ganglion, but this was brief compared with the effect on the response to preganglionic stimulation or on the response to acetylcholine.

Perhaps the most interesting result obtained by Bülbring was the demonstration that when the preganglionic fibres were stimulated the perfusion fluid leaving the ganglion was found to contain a substance having the properties of adrenaline. Thus the perfusate stimulated the heart of the frog, caused relaxation of the isolated rectum of the pigeon, and gave the fluorescence shown by Gaddum and Schild (36) to be characteristic of adrenaline. When samples of perfusate obtained during stimulation were compared with known adrenaline solutions by these three tests, the perfusate was found to have activity equivalent to the same concentration of adrenaline by each test, and this concentration was actually the concentration which when added to the perfusion fluid augmented the response to preganglionic stimulation.

Thus Bülbring was able to demonstrate that adrenaline could, by an action in the sympathetic ganglion, both augment and depress the effect of preganglionic stimulation, and both augment and depress the action of acetylcholine in the ganglion. Finally she showed that when preganglionic fibres were stimulated, not only acetylcholine, but also adrenaline was liberated in the ganglion, so that the resulting effect in the postganglionic fibre depended on the synergism between the two substances.

Parasympathetic effects. The demonstration of a synergistic relationship between adrenaline and acetylcholine at different positions in the central and peripheral nervous system, raises the question whether adrenaline affects those peripheral actions of acetylcholine which are readily paralysed by atropine, such as the action on the heart, the blood vessels, the intestines and glands like the salivary glands. So far as the heart is concerned there are no observations which indicate a synergism. On the blood vessels however Katz and Schwartz (37) obtained evidence respecting the influence of adrenaline on the constrictor reaction of the vessels of the rabbit's ear to acetylcholine. The vasoconstrictor action of acetylcholine, which is in some respects similar to that of nicotine, was first observed in the rabbit's ear by Reid Hunt (38). Others who have described similar effects include Fleisch (39), Hirose (40), Feldberg and Minz (41) and Brandt and Katz (42). Katz and Schwartz first observed the reaction of the vessels to acetylcholine, then perfused the ears with adrenaline (1 in 10^9 or in

10^{10}) for 10 to 20 min., and then washed out the adrenaline with Tyrode's solution. Dilutions of acetylcholine (1 in 10^4) which when continuously perfused through the ear had previously caused some dilatation, now caused powerful constriction. That is to say, Katz and Schwartz observed that when the vessels had been treated with adrenaline the constrictor action of acetylcholine was greatly enhanced. The dilator action of weaker solutions was converted by adrenaline to a diphasic reaction consisting of dilatation followed by constriction.

Bülbring and Burn (7) observed that the vasodilator action of acetylcholine in the perfused hindleg of the dog was greater when the arterial resistance was maintained at a given point by adrenaline, than when it was maintained at the same point by pituitary (posterior lobe) extract. The same was true for the vasodilator action of histamine, however, so that it was not an effect specific for acetylcholine. Turning to the salivary glands, Stavraky (43) has found that adrenaline, which in a dose of 0.01 mgm. does not cause salivation in the cat, greatly increases the secretion caused by pilocarpine, eserine or mecholyl. The action of pilocarpine is not an action exerted through the submaxillary ganglion, and this potentiation of pilocarpine by adrenaline must take place in the gland cells, and not at the synapse of the ganglion.

The basis of the potentiation. What is the basis of the potentiation of the action of acetylcholine by adrenaline? The potentiation of the action of acetylcholine by eserine is well-known, and depends on competition by eserine molecules for the enzyme cholinesterase. The recent work of Krayer, Goldstein and Plachte (44) suggests that cholinesterase destroys eserine, though this destruction takes place at a slower rate than that of acetylcholine. Has adrenaline any similar action? Is adrenaline an anticholinesterase? Some action of this kind has been described by Waelsch and Rackow (45) for the oxidation products of adrenaline. They have pointed out that both acetylcholine and adrenaline are substituted methylethanol amines, and that both compounds are N-methylated. Further they say that eserine contains an N-methyl indole portion of the molecule which relates its structure to that of adrenochrome, this being also a substituted N-methyl indole. They investigated the action of oxidation products of adrenaline on the cholinesterase in human serum and found that while the product obtained by oxidation with catechol oxidase was itself inactive, it had a strong inhibiting action when made alkaline. Solutions of adrenaline exposed to air in thin layers possessed increasing inhibiting action.

Ellis (46), however, has found no evidence for inhibition of cholinesterase by adrenochrome using pharmacological methods such as the leech muscle method and the frog's rectus abdominis method. Moreover an explanation based on an anticholinesterase action of adrenaline or its breakdown products would not cover all the facts. In denervated skeletal muscle adrenaline potentiates the action not only of acetylcholine but also of tetramethylammonium iodide. If effects involving prostigmine are also to be fitted into the picture, there must be an explanation of the potentiation by prostigmine of the constrictor action of adrenaline on the blood vessels of the dog. Nor does it seem easy to explain the

potentiation of the effect of prostigmine in skeletal muscle by adrenaline as an additional anticholinesterase action, since the effect of eserine in this tissue is increased very little by adrenaline.

The effects of adrenaline may be explained by altered permeability of cell membranes. Acetylcholine molecules before making contact with cell receptors may traverse a membrane first. If adrenaline facilitates this passage, a smaller percentage of molecules of acetylcholine may be destroyed by cholinesterase and a larger percentage may engage with cell receptors. But again this explanation would not cover tetramethylammonium iodide. More facts are needed.

DISCUSSION AND SUMMARY

The evidence which has been summarised in the foregoing review indicates that acetylcholine and adrenaline in the nervous system have a different relationship from that existing elsewhere. Whereas in the heart, the viscera and throughout the tissues innervated by the autonomic system these two substances have actions which are antagonistic, in the nervous system on the other hand, adrenaline, having little action of its own, exerts a powerful influence in modifying the action of acetylcholine. In general it can be said that in low concentrations adrenaline augments the effect of acetylcholine, while in higher concentrations it depresses it.

Evidence of this action has been obtained at more than one point of the neurones originating in the anterior horn of the spinal cord. In the presence of adrenaline, acetylcholine injected into the spinal cord causes a motor discharge which probably originates in the anterior horn cell itself. The transmission of impulses along the course of the nerve is greatly affected by adrenaline, or by stimulation of sympathetic fibres. The transmission may be increased as much as two or three times, or it may be depressed. This depression, occurring as it does after sympathetic stimulation, is possibly the physiological change underlying the condition in which an animal is paralysed by fear. Since recent evidence suggests that transmission along the course of nerve fibres involves the liberation of acetylcholine and its destruction by the cholinesterase which is present in the medullary sheath, the effect of adrenaline on transmission may be due to its interaction with acetylcholine in this process also.

At the neuromuscular junction one action of adrenaline has long been known, that in which transmission from the nerve to the muscle is improved by adrenaline or sympathetic stimulation when fatigue has first occurred; this was described for adrenaline by Gruber, and for sympathetic stimulation by Orbeli. A second action has now been demonstrated, namely, the potentiation by adrenaline of the action of prostigmine. It is certainly not easy to ascribe either of these effects to a simple potentiation of acetylcholine by adrenaline; the facts are not so uncomplicated as that; nevertheless a synergism between these two substances is likely to be part of the story.

The picture is most complete for the sympathetic ganglion. Evidence of various kinds indicated that adrenaline in low concentration improved ganglionic transmission and in high concentration depressed it. These indications have

now been superseded by Bülbring's demonstration of the phenomena in the perfused superior cervical ganglion. The transmission of submaximal stimuli applied to the preganglionic fibres is increased by small amounts of adrenaline and depressed by larger amounts. That this effect of adrenaline is due to its potentiation of the acetylcholine transmission is shown by the observation that when the ganglion is stimulated by the injection of acetylcholine into the perfusion fluid, the stimulant action is greatly enhanced by the injection of a small dose of adrenaline and depressed by a large one. Finally Bülbring has shown that adrenaline is liberated from the superior cervical ganglion when the preganglionic fibres are stimulated, so that actually in each ganglion provision is made for adrenaline to exert its potentiating action on the acetylcholine through which the transmission is effected. This strong evidence that such an interplay occurs at all normal times in each sympathetic ganglion is the best reason for expecting a similar interplay at those synapses in the central nervous system where acetylcholine transmits the impulse.

If the action of acetylcholine in the central nervous system, including the brain, is indeed modified by adrenaline, it is conceivable that this modification is the basis of changes in nervous reaction, and even in behaviour, which occur in emotional states when an abnormal concentration of adrenaline is present.

It has also been shown that large doses of adrenaline, injected at a given moment into the general circulation depress ganglionic transmission, so that splanchnic stimulation, for example, is less effective than before. This change, which does not occur during the steady continuous infusion of adrenaline into the blood stream, but only on the administration of single doses, must make the body less capable of maintaining a normal blood pressure, when a sudden stimulus, such as is provided by the crash in an accident, causes the liberation of adrenaline at one moment in the blood stream.

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THE INTERMEDIARY METABOLISM OF FATTY ACIDS

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SCOPE OF THE REVIEW. The review is confined to a presentation of the current views on the intermediary metabolism of the fatty acids, i.e., the chemical reactions, so far as they are known, involved in the partial or complete oxidation of fatty acids in mammalian organs. The essence of controversial aspects will be presented in some detail, with an evaluation of the evidence. The more recent papers on the subject will be reviewed against the background of old evidence.

Many subjects collateral to the main topic are excluded. Questions of digestion, absorption, transport and storage of fats are outside the scope of the review (Smith, 159; Bloor, 18; Longenecker, 114). The field of metabolism of phospholipids has recently been well covered (Chaikoff, 32; Sinclair, 158). Special topics such as the metabolism of branched fatty acids have also been recently reviewed (Carter, 30). The main work on the use of deuterium in the study of fat metabolism has been ably presented by Schoenheimer (145, 146, 147). The field of the hormonal regulation of fat metabolism will be discussed only incidentally. There are recent reviews (Ingle, 85; Houssay, 83). Lipotropism has been discussed by Best and Lucas (13) and also Crandall (38). There is also a recent discussion of lipocaic by Dragstedt (52). Ketosis in cattle and the fat metabolism of the mammary gland of the cow is discussed in an interesting series of papers by Shaw and his co-workers (152, 153, 154, 155, 156).

ENZYMES CONCERNED WITH FAT METABOLISM. The presence of enzymes activating fatty acid molecules or their intermediates in tissues is obvious, but in the main the demonstration of their activity has been in the intact animal or surviving intact tissue. Only a beginning has been made in the study of these enzymes free from cellular structure. In comparison with analogous field of carbohydrate intermediary metabolism, the questions of the types of chemical reactions catalysed, the problem of co-enzymes or other prosthetic groups, the study of possibly associated phosphorylations, etc., is still in its infancy.

Apparently the first enzyme concerned with fat metabolism demonstrated free from cellular structure was beta-hydroxybutyrate dehydrogenase; its action is now fairly well defined. Early observations by Wakeman and Dakin (182) showed that aqueous extracts of dog liver oxidized β-hydroxybutyrate to aceto-acetic acid, and they subsequently showed (183) that ground liver reduced aceto-acetic acid to β-hydroxybutyrate. Wishart (192), using alkaline phosphate extracts, demonstrated by the methylene blue technic the presence of this dehydrogenase in muscle as well as liver. These observations were confirmed by Rosling (144), as well as Thunberg (178). Banga, Laki and Szent-Gyorgyi (4) showed that the co-enzyme necessary for this action was the same as that for the oxidation of lactic acid. Later, Green (74) prepared the dehydrogenase from

pig heart and measured its redox potential. He showed that it is cozymase-I linked and that flavin, flavin-protein or adrenochrome are the necessary carriers for aerobic activity.

Kühnau (102) reported on the activity of an enzyme solution prepared from liver by extraction. He found that prolonged equilibration (20 hrs.) with β-hydroxybutyric acid resulted in a decrease of the substrate greater than that accounted for by the increase of aceto-acetic acid. By qualitative tests he reported the formation of aldol, 1,3, butyleneglycol, acetaldehyde, succinic, fumaric and malic acids as well as traces of acetic and pyruvic acid. These results lack confirmation and must be regarded with some reserve. Leloir and Muñoz (110) have prepared from liver by homogenization an enzyme which oxidizes butyric acid. The preparation is done under a continuous stream of oxygen, and at 0°C. to avoid over-heating, since the enzyme is otherwise rapidly inactivated. The resultant cell-free preparation rapidly oxidizes (25°C) butyric acid. Succinic, fumaric and citric acid added to the system increased the rate of disappearance of the butyric acid, particularly if the preparation had been inactivated by anaerobiosis during preparation. In a subsequent paper Muñoz and Leloir (135) described their experiments in more detail. Preparations partially inactivated by anaerobiosis are restored by the addition of heated liver extract, indicating the necessity for a co-enzyme. For full activity the presence of phosphate, fumarate, cytochrome C, magnesium (or manganese) and adenylic acid were necessary. Of a large number of substrates tested only butyrate, valerate, hexanoate, heptanoate, octanoate were appreciably oxidized. No significant activity was found with formate, acetate, lactate, pyruvate, propionate. The higher fatty acids brought about a decrease in oxygen uptake. Coupling with phosphorylations seems indicated since phosphate was necessary for full activity, and the inhibitors of phosphorylations (fluoride, iodoacetate) inhibited the oxidation. This system does not appear to be identical with that of Lang (v. infra) since it is unstable, and does not oxidize the higher fatty acids. The concomitant oxidation of fumarate was required to obtain maximum activity of the enzyme, indicating a possible involvement of the Krebs cycle. The precise product of oxidation remains to be determined. The authors surmised it to be β-hydroxybutyric acid (no aceto-acetic acid is formed) since approximately one molecule of oxygen is consumed per mole of butyric acid decrease.

Lang, in a series of papers (103, 104, 105, 106), described an enzyme obtained from rat liver by extraction with phosphate at pH 8. It acted as a dehydrogenase upon either odd or even fatty acids, activity increasing with the length of the acid chain. The system had little effect on unsaturated fatty acids and appears to be specific for the saturated higher ones. The products of oxidation were shown to be unsaturated fatty acids. A co-enzyme, which may be obtained from heated rabbit muscle extracts, appears necessary. This was shown to be muscle adenylic acid, that from yeast being inactive except at higher concentrations. Inosinic acid, adenosine triphosphate, and muscle adenosine in equimolar amounts are equally effective. Adenine is ineffective. The

product of the action of the enzyme upon stearic acid was shown to be oleic acid and not an alpha-beta unsaturated octodecenic acid.

Fontaine (66) has confirmed Lang's findings of a fatty acid dehydrogenase in liver which requires the presence of a coenzyme for activity. Quagriarello (139) reported the presence in bile of a dehydrogenase which is active in the presence of stearic acid causing an extra consumption of oxygen. A double bond is formed. The activity of the enzyme was inhibited by KCN, while carbon monoxide was without influence. Mazza and Stolfi (123) also studied this system and demonstrated the presence in liver extracts of an enzyme which oxidizes stearate in the presence of oxygen. They were unable to show its presence in muscles, kidneys, or pancreas. In further studies Mazza and Marfori (122) showed that the system brings about oxygen uptake, an action which is inhibited by carbon monoxide, indicating a possible involvement of the cytochrome system. Diaphorase, glutathione, diphosphopyridine nucleotide, and adenylic acid were without influence. Since the system was sensitive to cyanide the authors concluded that it contains a heavy metal. The action was much more rapid upon fatty acids saturated or unsaturated, containing 12 or more carbons. The authors made the interesting observation by means of spectroscopic studies that the oxidation was in the alpha-beta position. Quagriarello (140) working with the same system reported that stearic and oleic acids were oxidized with equal rapidity.

Behrend (11) by glycerol extraction of pancreas obtained an enzyme system which acted upon tristearin with the formation of double bonds. Adipose tissue itself has been shown to contain fatty acid dehydrogenases by Shapiro and Wertheimer (151) who extracted one which depended for its activity upon adenylic acid and inorganic phosphate. The optimum pH was 8, and the action was greatest with the natural long chain acids, decreasing significantly with diminishing chain length. Neutral fat was not acted upon, but phospholipids were. Of the non-fat substrates only succinate was oxidized with rapidity comparable to that with palmitic and stearic acids. The enzyme, or a similar one, was also demonstrated in liver, muscle, heart, and testes. Iodo-acetate, fluoride, malic acid, pyrophosphate and benzoate had no effect, whereas exposure to oxygen appears to inhibit the enzyme activity. Annau, Eperjessy, and Felszeghy (1) also described a fatty acid dehydrogenase which they obtained from beef liver. This, as demonstrated by the methylene blue technic, oxidized lecithin, stearic and palmitic acids. An activator—which was obtained from boiled liver extracts—appeared necessary for full activity. Extended work on isolation, purification and identification showed that the activator was mainly hypoxanthine. In confirmation, xanthine and hypoanthine were found to be effective as activators. Apparently the enzyme system does not oxidize xanthine for the authors state that "das Enzyme ist nicht Xanthineoxidase." This unusual rôle of purines as co-enzymes is not elaborated upon, and the possibility that hydrogen peroxide formed upon oxidation of xanthine produces secondary oxidation effects upon the fatty acids was not discussed. Presumably this possibility was excluded by the authors.

Lehninger (107) made an attempt to obtain by extraction from rabbit kidney and muscle an enzyme system free from cells and active in the oxidation of acetoacetate. Apparently feeble but definite activity was found in his preparations. Acetoacetate disappeared, the end products not being identified except that approximately 25 per cent of the acetoacetate was recovered as acetic acid. The enzyme was unstable being inactivated in 1 hour at 38°C, hence further purification was difficult. Although not established, a cofactor appears necessary.

The information concerning these enzymes is summarized in table 1.

TABLE 1

Cell free enzyme systems concerned with fat metabolism

ORGAN	SUBSTRATE	PRODUCT OF OXIDATION	COENZYME OF ACTIVATOR	REFERENCE
Liver muscle....	β-hydroxybutyrate	Acetoacetate	Cozymase I	Wakeman and Dakin, 182–3; Green, 74
Liver.....	β-hydroxybutyrate	Acetoacetate misc. oxid. products	Not stated	Kühnau, 102
Liver.....	Lower fatty acids	β-hydroxy-butyrate	C_4 dicarboxylic acids, adenylic acid phosphate Mg (Mn)	Leloir and Muñoz, 110, 135
Liver.....	Higher saturated fatty acids	Unsaturated acids	Adenylic acid	Lang, 103–106
Bile.....	Stearic, oleic acids	Unsaturated acids	Not stated	Quagriarello, 139
Liver.....	Palmitic, stearic acids; lecithin	Reduction products	Hypoxanthine	Annau et al., 1, 2
Kidney muscle....	Acetoacetate	Not known	Not stated	Lehninger, 107

It is too early to evaluate the significance of these studies on isolated fatty acid oxidizing enzymes. Aside from the well worked out case of β-hydroxybutyrate dehydrogenase, the evidence is conflicting. All of the preparations used were impure, and in most cases a co-enzyme appears to be necessary for activity. At least one system was shown to be concerned with the oxidation of the low molecular weight acids with the production of β-hydroxybutyrate. This was found in the liver only, whereas the others were more widespread and were active upon the higher acids, the product of oxidation being a desaturated acid. In some cases adenylic acid appeared to be the co-enzyme whereas in others no co-enzyme has been demonstrated.

Nevertheless, an encouraging beginning has been made upon a subject which will require enormous labor before any clarifying generalizations can be made.

KETONE BODY PRODUCTION. It is becoming increasingly clear that the formation of ketone bodies (acetoacetate and β-hydroxybutyrate) in the liver is an important initiating step in the catabolism of fatty acids for the energy requirements of the peripheral tissues.

Site of formation. It is generally agreed that the liver is the sole site of ketone formation. Attempts to show extra-hepatic ketone formation have failed. Eviscerated preparations from fasted or diabetic animals show a steady fall of blood ketone body concentrations. Mirsky (129) showed that anterior pituitary extracts which were actively ketogenic in the intact animal had no effect upon blood ketones in the eviscerated preparation. Harrison and Long (78) found, in severe ketotic states, no ketones whatever in muscle water even in the presence of high blood ketones, indicating a complete oxidation rather than a production of ketone bodies at this site.

However, Jowett and Quastel (89) found a small production of ketone bodies using kidney, spleen and testis in vitro.

Precursors. It has long been recognized that the chief formation of the ketone bodies is by partial oxidation of the fatty acids in the liver. Certain of the amino acids are known to be ketogenic and they are listed by Shaffer (149) as follows: leucine, tyrosine and phenyl alanine. Isoleucine (Butts, Blunden and Dunn, 26) also is ketogenic. However, the amounts derived from protein are relatively small. Carbohydrate or any of its intermediates as a possible source of the ketone bodies is generally excluded. Some caution must be exercised on this point, however, in view of Krebs' (97) evidence from experiments with liver in vitro that acetoacetate may arise from pyruvate according to the reaction:

2 Pyruvic acid + O_2 = Acetoacetic acid + $2CO_2$ + H_2O. Whether this pathway from carbohydrate to ketone bodies plays any significant rôle in the problem of ketosis is undetermined.

CHEMICAL MECHANISM OF KETONE PRODUCTION FROM FATTY ACIDS. Three theories have been presented to explain the production of ketone bodies by partial oxidation of fatty acids in the liver. Viz:

1. Successive beta oxidation.
2. Multiple alternate oxidation.
3. Successive beta oxidation + condensation.

1. *Successive beta oxidation.* The original experimental work of Knoop (92) upon which the theory was based was confined to the study of phenyl-substituted fatty acids in which the side chain contained 5 carbons or less. When these acids were fed, the nature of the phenyl residue excreted clearly showed that these acids were oxidized at the beta carbon, viz:

$$\mathrm{Ph\cdot CH_2CH_2\cdot COOH} \xrightarrow[O_2]{} \mathrm{Ph\cdot CO\cdot CH_2\cdot COOH} \rightarrow \mathrm{Ph\cdot COOH} + \mathrm{C_2\ unit}$$

$$\mathrm{Ph\cdot CH_2CH_2\cdot CH_2\cdot COOH} \xrightarrow[O_2]{} \mathrm{Ph\cdot CH_2\cdot CO\cdot CH_2\cdot COOH} \rightarrow$$

$$\mathrm{PhCH_2\cdot COOH} + \mathrm{C_2\ unit}$$

The β keto acids with 3 or 4 carbons in the side chain initially formed were presumably further degraded by the splitting off of a two carbon unit, the fate of which was never determined satisfactorily. The phenyl residue, either benzoic or phenyl acetic acid, underwent a secondary reaction with glycine forming hippuric or phenaceturic acid. But in the case of valeric acid, the longest fatty acid which he studied, Knoop concluded that there was both beta and delta oxidation, viz:

$$\mathrm{Ph \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH} \xrightarrow[O_2]{}$$

$$\mathrm{Ph \cdot CO \cdot CH_2 \cdot CO \cdot CH_2 \cdot COOH} \xrightarrow[H_2O]{O_2} \mathrm{Ph \cdot COOH} + 2C_2 \text{ units(?)}$$

He made no assumptions concerning the possible splitting off of a two carbon unit or whether the oxidations were successive or simultaneous. This classical work has been amply confirmed, and there can be no doubt that beta oxidation not only of short fatty acids which may be used experimentally, but also of the naturally occurring long fatty acids, occurs in the body. For example, Stetten and Schoenheimer (172) using deuterium showed that stearic acid can be degraded to a small extent to palmitic, lauric and myristic acids. The recent work of Bloch and Rittenberg (17) (v. infra) on the acetylization of foreign amines may also be mentioned. The beta oxidation hypothesis has continued to stand upon firm ground, but difficulties arose when a specific application of it was developed which sought to explain the entire catabolism of fatty acids. Dakin (45) from his experiments became convinced that the acid: $\mathrm{C_6H_5CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH}$ "undergoes oxidation in the body in such a way that four carbons are removed from the side chain in two pairs. . . . " This process "may be termed successive beta oxidation" and he saw "no reason to suppose that it is not a general biochemical reaction" and "that the catabolism of a fatty acid group $\mathrm{CH_3 \cdot (CH_2)_n \cdot COOH}$, is effected by the successive removal of two carbon groups at a time." The successive beta oxidation hypothesis came to mean that each molecule of fatty acid is oxidized through a succession of fatty acids each shorter by two carbon atoms than its immediate precursor, viz:

$$\mathrm{CH_3 \cdot (CH_2)_n \cdot CH_2 \cdot CH_2 \cdot COOH} \xrightarrow[O_2]{}$$

$$\mathrm{CH_3 \cdot (CH_2)_n \cdot CO \cdot CH_2 \cdot COOH} \xrightarrow[H_2O]{O_2}$$

$$\mathrm{CH_3 \cdot (CH_2)_n \cdot COOH} + C_2 \text{ unit. Etc.}$$

Finally, one molecule of butyric acid remains which in turn is oxidized to acetoacetic acid. In other words, each fatty acid molecule yields only one molecule of acetoacetic acid, the balance being converted into two carbon compounds presumably acetic acid.

This theory despite its patent defects remained in almost unchallenged ascendancy for a period of forty years, and the reasons are not far to seek: 1, when com-

bined with the theory of obligatory ketone body-carbohydrate oxidation (q.v.) it explained the ketonuria in diabetes and the marked recession of ketosis following the resumption of carbohydrate oxidation; 2, it permitted the under-utilization school of diabetic students to explain how the complete diabetic, who was losing about 80 per cent of the energy from protein as carbohydrate or ketone bodies, and 100 per cent of that from carbohydrates, could still exist. For there was still available the energy derivable from the hypothetical two carbon unit, presumably acetic acid, split off from fats.

Multiple alternate oxidation. But the weaknesses of the successive beta oxidation theory which were apparent on closer inspection eventually brought about its dissolution. Though vigorously sought for, the hypothetical acetic acid formed was never demonstrated (Embden and Michaud, 63; Toenniessen and Brinkman, 179; Stadie, 169). Furthermore, the hypothetical intermediate acids with less than 12 carbon atoms were never found in tissues or fat depots of the mammalian organism (36). Small amounts of myristic and lauric were found but none with fewer carbon atoms. On the basis of these and other considerations, Hurtley (84) and later Jowett and Quastel (88) proposed the multiple alternate oxidation hypothesis. According to this the fatty acids are oxidized simultaneously along the whole length of the fatty acid chains at alternate carbons with the result that the entire molecule is converted into ketones, viz:

$$CH_3 \ldots CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \ldots COOH + O_2 = CH_3 \ldots CH_2CO \cdot$$

$$CH_2 \cdot CO \cdot \cdot CH_2 \cdot CO \cdot CH_2 \cdot CO \cdot \cdot COOH = n\ (CH_3 \cdot CO \cdot CH_2 \cdot COOH)$$

A typical fatty acid such as palmitic would thus yield 4 ketone molecules rather than 1. Viz:

$$C_{16}H_{32}O_2 + 5\ O_2 = 4C_4H_8O_3$$

The evidence supporting this hypothesis accumulated. Liver slices when equilibrated in the presence of various fatty acids (C_6–C_{10}) were found to give higher amounts of ketone bodies in comparison to that formed from the lower ones, a fact which could be explained only when it was assumed that more than one ketone molecule was derived from each fatty acid molecule (Jowett and Quastel, 88). Leloir and Muñoz (110) also studied ketone formation by liver slices in the presence of added fatty acids. Octanoic acid as compared to butyric gave amounts of ketone formation in excess of the 1:1 ratio. On feeding salts or ethyl esters of fatty acids up to C18 to fasting rats, Deuel and his co-workers (27, 47) found so much higher relative rates of ketone excretion from the higher fatty acids that it appeared unlikely that the formation of only one mole of ketones per mole of fatty acids would account for their findings. Blixenkrone-Moeller (14) determined the oxygen uptake and ketone production of perfused diabetic cat livers. He found a low O_2 to ketone ratio and he concluded that practically the entire molecule of fatty acid was converted to ketone bodies without the production of any two carbon residue such as acetic acid. Stadie (166), using liver slices from diabetic cats, found the values: oxygen consumption

to ketone formation given in the table 2. When the total oxygen uptake was corrected for carbon dioxide formation and for the observed deamination of amino acids, the observed ratio: oxygen ketone formation did not differ significantly from that required by the multiple alternate oxidation hypothesis. In later work Stadie and his co-workers (169) by a method capable of detecting very small amounts of acetic acid were unable to find a trace of acetic acid formation by liver slices which were actively producing ketone bodies. In addition, the formation of any hypothetical intermediary acids (C_4 to C_{10}) was excluded. Further, balance studies on fatty acid decrease compared to ketone body increase showed that all of the former would be accounted for by ketone formation, a finding in accordance with the multiple alternate oxidation theory.

Beta oxidation-condensation theory. The summation of this evidence appears to be convincing proof that the major portion of fatty acid oxidation in the liver,

TABLE 2

The oxidative metabolism of liver slices from six depancreatized cats (Stadie, 166)

OXIDATIVE PROCESS	MEAN OXYGEN UPTAKE MICROMOLES/GM./HR.
Deamination of amino acids calculated from urea and NH_3 formation	8.2 ±1.2
Carbon dioxide formation	28.0 ±5.0
All other oxidations, calc. by difference	51.3 ±6.5
Observed oxygen uptake	87.5 ±4.0
Ketone formation, observed	46.5 ±4.0
Corrected oxygen to ketone ratio observed	1.10 ±0.12
Calculated ratio for palmitic acid:	
Multiple alternate oxidation	1.25
Successive beta oxidation	6.5

at least that concerned with ketone production, occurs in such a way that the initiation of oxidation upon a given molecule proceeds to completion with great rapidity so that the entire molecule is oxidized to ketone bodies. No intermediary fatty acids are formed nor does any 2 carbon unit accumulate. This is not to imply that beta oxidation, in the original meaning of Knoop, does not occur, for the recent work of Stetten and Schoenheimer (172) proves that a minimal amount of step-wise degradation occurs. Nor does it exclude the possibility that fatty acids may, to a certain extent, yield acetic acid, as the work of Bloch and Rittenberg (17, infra) has amply shown. But the implication of the successive beta oxidation hypothesis as later developed and so firmly entrenched in biochemical thinking that large amounts of intermediary fatty acids down to butyric acid are formed in the liver together with acetic acid available as a peripheral fuel can no longer be held.

The original postulation of the multiple alternate oxidation hypothesis assumed

no detailed mechanisms by which the oxidation was brought about. However, mechanisms became read into the original hypothesis and it was implied by many that long chain polyketo acids were formed which were then split into four carbon units which then formed ketone bodies. But certain findings were incompatible with this conception. How could fatty acids with carbons not an even multiple of 4 be entirely converted into ketones? Only by assuming that the extra C_2 units were converted to ketones. In particular, caproic acid (C_6) was found to yield a greater amount of ketones than butyric acid (Deuel et al., 47). Caprylic acid is known to be glucogenic and to a certain extent ketogenic (Jowett and Quastel, 88) (Leloir and Muñoz, 110). If these transformations occur simultaneously some such reaction as:

$$C_5H_{10}O_2 \xrightarrow[H_2O]{O_2} C_3 \text{ unit} + C_2 \text{ unit} \rightarrow \text{Glucose } (C_6) + \text{ketone } (C_4)$$

would again imply the formation of ketone bodies from a two carbon unit. MacKay (115) proposed that these difficulties of the multiple alternate oxidation hypothesis be resolved by assuming the following mechanism: by successive beta oxidation the fatty acid molecule is split into 2-carbon units. But whatever their nature, these are re-assembled in *statu nascendi* into ketone bodies. The essential implication of the multiple alternate oxidation hypothesis is retained, that is, whenever oxidation is initiated on a given fatty acid molecule it proceeds practically instantaneously at alternate carbons along the entire chain to complete disruption into ketones somewhat like the successive explosions of a pack of fire-crackers. The sole difference between the two hypotheses is that fatty acids are split, on the one hand, into four carbon units forming ketone bodies, and, on the other, into two carbon units which are at once reassembled into ketones. The physiological significance of the two hypotheses is the same, but widely at variance with the successive beta oxidation hypothesis.

This proposed mechanism became known as the beta-oxidation-condensation hypothesis. MacKay (115) urged that the well known conversion of acetic acid to ketone bodies was proof for the beta-oxidation-condensation hypothesis, but this fact has been just as eloquently used by Friedemann (69 infra) to confute the original application of beta oxidation to fatty acid metabolism.

Direct proof came from the experiments of Weinhouse, Medes and Floyd (187). They equilibrated liver slices of rats with octanoic acid containing heavy carbon in the carboxyl group. The acetoacetic acid formed was broken down into acetone and CO_2 and the heavy carbon in these fractions was determined. Their results are shown in table 3. The observed and calculated values for beta oxidation-condensation agreed, and the authors concluded that in the liver oxidation of octanoic acid proceeds by mechanism involving splitting into 2 carbon fragments which condense to the ketone bodies. These interesting experiments give strong support to the beta oxidation-condensation hypothesis.

To emphasize the difficulty of determining the fine details of the mechanism of fatty acid oxidation, it must be remembered that Weinhouse's experiments

could just as well be explained by another hypothesis. If there exists an equilibrium between a two-carbon unit and acetoacetate, e.g.,

$$2CH_3 \cdot COOH \rightleftharpoons CH_3 \cdot CO \cdot CH_2 \cdot COOH + H_2O$$

an assumption supported by the well-known fact that acetic acid is easily converted to ketone bodies (Swenseid, 175), then a random distribution of the heavy carbon in the carbonyl and carboxyl groups would occur irrespective of the mechanism of oxidation of the original octanoic acid molecule.

To emphasize further the difficulties involved, the experiments of Morehouse and Deuel (133) must be discussed. After feeding α-β dideuterio caproate to

TABLE 3

Comparison of observed values of C_{13} distribution in acetoacetic acid predicted by various theories of ketone body formation (Weinhouse, Medes and Floyd, 187)

	ATOM PER CENT C^{13} EXCESS	
	Carbonyl group	Carboxyl group
Observed	0.84	0.85
Calculated:		
Beta oxidation-condensation	1.10	1.10
Multiple alternate oxidation	0.00	2.20
Successive beta-oxidation	0.00	0.00

rats they obtained 6.4 atom per cent of deuterium in the excreted β-hydroxybutyric acid, whereas with β-γ di-deuterio caproate they found 10.9 atom per cent. The following schema illustrates the possibilities involved:

	Intermediates	Possible deuterio β-hydroxybutyric acid	Expectancy in deuterio β-hydroxy butyric acid
Beta oxidation			
—C—C—C—CD—CD—CO	—C—CO—C—CO— —CD—CO		None
—C—C—CD—CD—C—CO	—C—CO—Cd—CO —C—CO	—C—CO—Cd—CO—	Trace
Beta oxidation-condensation			
—C—C—C—CD—CD—CO	—C—CO— —CD—CO—	—CD—CO—Cd—CO	Equal
—C—C—CD—CD—C—CO	—C—CO— —CD—CO	—CD—CO—Cd—CO	
Beta-delta oxidation			
—C—C—C—CD—CD—CO—	—C—CO— —C—CO—Cd—CO—	—C—CO—Cd—C—	Trace
—C—C—CD—CD—C—CO—	—C—CO— —CD—CO—C—CO—	—CD—CO—C—CO—	Greater

According to Morehouse (132) only traces of deuterium are retained in deutero β-hydroxybutyric acid when it is in the alpha position; accordingly deuterium in this position is indicated by "d." The experimental findings are not in complete accord with any of the hypotheses illustrated. Beta oxidation appears definitely ruled out. Beta oxidation-condensation requires equal quantities of retained deuterium in the beta hydroxybutyric acid forming from the two caproates, contrary to the findings. The results are in best accord with beta-delta oxidation. This would mean that during the oxidation of the caproic acid a four carbon unit has retained its integrity, a conception implicit in the multiple alternate oxidation hypothesis. To explain the finding of finite amounts of deutero-beta hydroxybutyric acid from alpha-beta di-deuteric caproic acid, it is conceivable that beta-oxidation-condensation and multiple alternate oxidation occur simultaneously, the latter predominating.

Another difficulty might be mentioned. Bloch and Rittenberg (17) found no significant labelled acetylization following ingestion of 9 to 10 di-deuteric stearic acid, a result which they attributed to deposition rather than catabolism of the labelled fatty acid. However, it is also possible that with the higher fatty acids beta oxidation is minimal, a supposition in accord with the experiments of Stetten and Schoenheimer already quoted.

In summary it may be concluded that beta oxidation in the strict sense initially defined by Knoop, is a real phenomenon; that the special application to explain the entire oxidative catabolism of fatty acids generally known as the successive beta oxidation hypothesis must be discarded; that ketone bodies are formed in the liver by conversion of the entire molecule of fatty acid. The evidence is good that two carbon units are formed as intermediate which become stabilized as ketones, in acetyl groups, in cholesterol, or other undetermined forms. But in some cases four carbon units also form. Hence, at the present moment, it is impossible to generalize as to whether one or the other mechanism is the exclusive or predominating one.

Ketone formation from acetic acid. In discussion of theories of ketone production acetic acid occupies a prominent place. The original investigation of Loeb (113) and Friedemann (69) by liver perfusion experiments showed that the addition of acetic acid was followed by abundant ketone formation. The supposition is that it is converted to ketone bodies presumably by the reaction:

$$2CH_3 \cdot COOH \rightleftharpoons CH_3 \cdot CO \cdot CH_2 \cdot COOH + H_2O$$

Acetic acid is also converted to ketones by liver slices (88, 111), and when fed to the intact animal ketone body production is increased (116) as determined by urine and blood measurements. By the use of acetic acid containing heavy carbon in the carboxyl group, it has been shown by Swenseid, Barnes, Hemingway and Nier (175) that the production of ketones may, in part at least, be due to condensation. However, analysis of their data shows that a relatively small proportion of ketone bodies produced originated from the heavy carbon acetic acid fed. The balance must have been formed by some other mechanism initiated by the ingestion of the acetic acid.

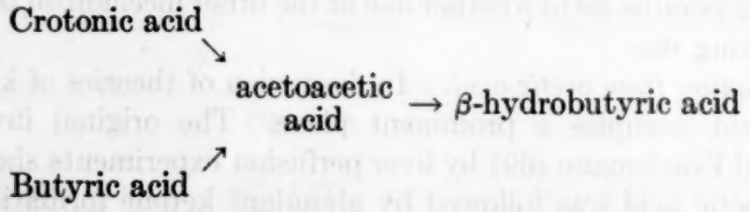
Krebs and Johnson (100) suggested the possibility that acetopyruvate is an intermediary in the formation of ketones from acetic acid by way of condensation with pyruvate, viz.:

$$CH_3 \cdot COOH + CH_3 \cdot CO \cdot COOH - H_2O \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot CO \cdot COOH$$
$$\rightarrow CH_3 \cdot HCOH \cdot CH_2 \cdot COOH + CO_2$$

They found that liver, muscle, kidney, testis, and brain metabolize acetopyruvate, and that in the liver acetoacetate was formed under aerobic, and β-hydroxybutyrate under anaerobic conditions. Further, ketone bodies were formed more rapidly from acetopyruvate than from acetic acid or pyruvic acid. However, Weinhouse et al. (187) concluded that their experiments rule out this mechanism. Lehninger (108) in further studies found that acetopyruvate was rapidly metabolized by rats, being converted to ketone bodies and possibly pyruvate and glucose. The paradoxical fact was found that whereas it protected against insulin hypoglycemia it did not lead to the formation of glycogen in the liver. Lehninger supposed two different mechanisms of breakdown: (1) to ketone bodies in the state of ketosis and (2) to pyruvic acid to glucose in the state of hypoglycemia, a possibility realized in the case of isoleucine which as Butts et al. reported (26) is both ketogenic and glucogenic.

LOWER FATTY ACIDS. Much experimental work having been done on the lower fatty acids, they deserve some special discussion.

In a series of papers (87, 88, 89) Jowett and his co-workers reported experiments using tissue slices (kidney and liver). Rates of formation and the effects of inhibitors brought them to the conclusion—opposed to the then generally accepted view—that butyric acid is oxidized directly to acetoacetic acid which in turn is transformed in part to β-hydroxybutyric acid. Crotonic acid is not an intermediate. Viz.:



The authors gave an interesting example of competitive inhibition of the fat oxidative enzyme system: that shown by butyric and hexanoic acid (88). Acetoacetate production by livers is always greater with the former. With both substrates present ketone production falls to that usually obtained with hexanoic acid indicating that it has displaced butyric from the enzyme. The same phenomenon was observed with crotonic and butyric acids. Acetic acid transformation to acetoacetate apparently involves a different system from that oxidizing the acids because when acetic acid and butyric acid are together as substrates, the acetoacetate formation is then additive. Blixenkrone-Moeller (15) reported that butyric acid but not β-hydroxybutyric acid was converted to glucose in the perfused cat liver as judged by the occurrence of a high D-N ratio. He concluded that β-hydroxybutyric acid is not an intermediate in the

pathway to carbohydrate. Caprylic acid, on the other hand, yielded ketone bodies only. He assumed that part of the oxidation of butyric acid is by gamma oxidation to succinic acid which is then transformed to glucose. Kleinzeller (91) studied the metabolism of a large number of four carbon monocarboxylic acids. Of these butyric, crotonic, vinylacetic, β-hydroxybutyric, γ-hydroxybutyric, dl-α γ-dihydroxybutyric were readily oxidized by kidney slices. Vinylglycollic, α-hydroxybutyric, tetrolic, dl-β γ dihydroxybutyric and tetronic acids were not appreciably oxidized. He concluded from his data on rates of oxidation that β-hydroxybutyric acid is not an intermediary in the oxidation of butyric acid in the kidney, thus indicating that the metabolic pathway in this organ is different from that in the liver where, as is well known, butyric acid readily yields β-hydroxybutyric acid. Vinyl acetic acid ($CH_2{:}CH \cdot CH_2 \cdot COOH$) was found to be metabolically very active and similar to butyric acid in its reactions, a fact leading Kleinzeller to suggest that it is an intermediate in the metabolism of butyric acid. He further confirmed Jowett and Quastel (88) in finding that malonate inhibited the oxidation of all active substrates enumerated.

Lipmann and Perlman (112) confirmed Quastel's opinion that β-hydroxybutyrate does not form from crotonic acid by the addition of the elements of water in spite of the existence of fumarase or aconitase in tissues which brings about the transformation of unsaturated polycarboxylic acid to the corresponding hydroxy acids. For they were unable to show any formation by liver slices of β-hydroxybutyrate from crotonate anaerobically. β-hydroxybutyrate is the reduction product, not the precursor of acetoacetate. In discussing desaturation as an intermediary step in fat oxidation they suggested that desaturation may be associated with phosphorylation, viz.:

$$—CH{:}CH— + H \cdot OPO_3H_2 = —C(OPO_3H){:}CH— + 2H$$

Through this reaction an energy rich phosphate bond is formed which can be utilized in the final reaction leading to the formation of a keto compound: viz.

$$—C(OPO_3 \cdot H_2){:}CH— + H_2O = —C(:O) \cdot CH_2— + H_3PO_4 + 11 \text{ Kgcal.}$$

Further work on the possible phosphorylation of fatty acids during oxidation was reported by Lehninger (110). Using rat liver homogenates he found that there was a considerable extra oxygen uptake with normal saturated fatty acids having 4 to 18 carbons when cytochrome C and adenosine polyphosphate were present. His experiments suggested that there is an intermediate formation of acyl-phosphate because synthetic acyl phosphates (C 8–16) apparently did not require ATP for oxidation.

Cohen (35) studied ketone formation by rat liver slices. He used a large variety of fatty acids and their homologues, viz.: normal fatty acids (C_2 to C_6), amino acids, α-hydroxy and α-keto acids (C_4, C_5, C_6), many types of 4, 5, and 6—carbon fatty acids. In an interesting discussion he pointed out that a definite chemical grouping is necessary for oxidation by what he calls the beta-oxidase system. In skeleton form the specific group has the structure —CH=CH·CO—. On the basis of this hypothesis he explains the results obtained with his divergent

substrates. The glucogenesis and slight ketogenesis of the higher odd numbered fatty acids can also be thus explained. In this case, competitive inhibition by glucose or a glucose-precursor causes inhibition of ketone formation.

Proportion of acetoacetate: beta hydroxybutyrate. Since the interconversion of the ketone bodies one into another is well established (cf. Enzymes) the liver produces a mixture of the two. The ratio between them varies considerably. In normal and diabetic cat liver slices, Stadie, Zapp and Lukens (167) found a ratio of approximately 1:1. Crandall (37) found a much greater variation in dogs; the per cent of β-hydroxybutyrate to total ketones varied from 35 to 93 per cent; his average value was 64 per cent. He found that the ratio in the liver was the same as that found in the blood. Stark and Somogyi (170, 171) defined the "beta ratio" as that between the β-hydroxybutyrate and total ketones in the blood. They made some interesting observations respecting it, and also the distribution ratio between cells and plasma in diabetic patients. Those livers which retain appreciable degree of carbohydrate utilization respond as do normals to glucose feedings: the beta ratio decreases consistently, and drops to zero during the 3rd and 4th hours. On the other hand, in diabetic patients whose livers are unable to utilize significant amounts of carbohydrate, the ketonemic level after glucose feeding is maintained for a longer time, and there is no significant change in the beta ratio of the blood. In patients with severe ketosis, glucose feeding has no effect until, by the injection of massive doses of insulin, carbohydrate utilization by the liver is restored; the normal sequence of events then follows. The urine in general follows the course of the blood, but is not so reliable an index. In ketosis in man and dogs from various causes, Friedman (68) found an average ratio of hydroxy to keto acid of about 2. The factors responsible for variations in the ratio in blood and urine are discussed in detail.

On the basis of studies with liver slices Annau, Eperjessy and Zathureczky (2) divide the fatty acids into two groups: 1, palmitic, stearic, oleic, and the lower fatty acids which form ketones upon oxidation in the liver; 2, fatty acids with 2, 3 or 4 double bonds, i.e., linoleic, linolenic, arachidonic, whose oxidative breakdown is different from the first group, since they cause an increase rather than a decrease in the R.Q. of liver slices.

Lag in ketone production by liver. Since it appears to be a function of the liver to oxidize partially fatty acids for peripheral utilization, the time relations between this process and the need of the periphery become important. Normally the production of ketones by the liver is minimal, but, unlike the rapid mobilization of hepatic carbohydrate, the liver appears unable to form ketones quickly for peripheral needs. For example, the fasting angiostomized dog shows a relatively slow increase in the ketone body production of the liver, maximum levels being reached only after 2 to 4 days (Crandall, Ivy and Ehni, 41). During this lag period much of the necessary fat calories must be furnished by direct oxidation of fats in the periphery. In fasting man (Crandall, 38) the lag appears to be shorter, maximum blood ketone levels being reached in 39 hours. However, the effect of heavy exercise in rats is reported by Drury, Wick and MacKay (54)

to be accompanied by an initial drop in blood ketones followed in 2 to 3 hours by a high level indicating a quicker response in this species.

This question of lag becomes important in the problem of the mode of oxidation of fats by muscle (q.v.).

Miscellaneous Factors. Endocrine relations. Mirsky (126) reported the interesting observation that the ketonemia regularly following the injection of anterior pituitary extract was suppressed in rabbits by the simultaneous administration of insulin. This effect was attributed to the specific action of insulin upon the liver in preventing hepatic glycogenolysis.

Acidosis and alkalosis. The well-known ketogenic effect of alkalosis has been studied by MacKay, Wick, Carne and Barnum (120). Using the blood level of ketones in fasting rats as the criterion, they found that acid administration increased liver glycogen, daily excretion of nitrogen and sulphur, while alkalosis leads to opposite findings. They concluded that the corresponding increased or decreased glucose formation from protein explains the resulting effects upon blood ketones.

Ketone formation and ammonia. The well-known effect of increased ketone production by liver slices in the presence of ammonia (56) continues unexplained. Linoleic acid was reported to decrease the ketogenic action of ammonium salts (Annau, Eperjessy and Zathureczky, 2).

Omega oxidation and the catabolism of dicarboxylic acids. The idea that the fatty acids are catabolized by way of a preliminary oxidation of the terminal methyl group with the production of dicarboxylic acids as intermediaries has not escaped advocacy. The chief exponent of this theory of omega oxidation is Verkade (180), who has set forth his views in detail in the reference cited. Following the accidental discovery of diaciduria upon the administration in man of triundecylin (C_{11}), he elaborated the theory illustrated by the following schema:

$$\begin{array}{ccc} CH_3\cdot(CH_2)_n,COOH & \xrightarrow{\text{Omega oxidation}} & HOOC\cdot(CH_2)_n,COOH \\ \downarrow \text{Beta oxidation} & & \downarrow \text{Beta oxidation} \\ CH_3\cdot(CH_2)_{n-2}\cdot COOH + C_2 \text{ unit} & \xrightarrow{\text{Omega oxidation}} & HOOC\cdot(CH_2)_{n-2}\cdot COOH + C_2 \text{ unit} \end{array}$$

Monocarboxylic fatty acids are oxidized on the terminal methyl group with the formation of a dicarboxylic acid. Subsequently by bilateral beta oxidation the dicarboxylic acid is degraded two carbon units at a time with the formation of acetic acid and a dicarboxylic acid containing two carbons less than the precursor. Or, the original fatty acid may undergo beta oxidation, the resultant degraded monocarboxylic acid being then oxidized by omega oxidation with the formation of a dicarboxylic acid containing two carbons less than the original. These reactions continue until a four carbon dicarboxylic (succinic) acid is produced. A direct pathway for the conversion of fatty acids to carbohydrate is thus provided since all four-carbon dicarboxylic acid are carbohydrate precursors.

The evidence upon which this hypothesis is based is devious and Verkade's summarizing lecture should be consulted for the finer details:

1. In man, following ingestion of monocarboxylic acids, di-acids are found in the urine. But, this property is confined to the lower ones in the series: viz.

MONOCARBOXYLIC ACID-CARBONS	DIACIDOCENIC PROPERTIES
8	Weakly
9	Weakly
10	Strongly
11	Strongly
12	Slightly or none
13	None

In dogs, however, the diacidogenic properties of C_7 to C_{11} acids was difficult or impossible to demonstrate. In explanation of this difference, the difficulty of producing ketonuria in dogs is cited, and the lack of diacidogenesis is "possibly related and to be expected with the carnivora."

2. Methyl oxidation is well described in the literature and di-carboxylic acid formation is simply one example of it. The probable mechanism is over some route such as

$$—CH_3 \rightarrow —CH_2— \rightarrow —CHO \longrightarrow —COOH$$

3. Although the lower dicarboxylic acids (C_6–C_{10}) when administered or injected are largely excreted unchanged, the higher ones are apparently catabolized since they fail to appear in the urine: e.g.,

DICARBOXYLIC ACID FED (DOG)	% EXCRETED UNCHANGED
C_6	58
C_8	45
C_{10}	33
C_{11}	17
C_{13}	±0
C_{16}	0

Apparently the combustibility of these acids by the dog rapidly increases on ascent of the homologous series. The relative non-combustibility of the lower dicarboxylic acids, which are presumably intermediaries in the catabolism of the higher ones, is explained with difficulty.

4. Following ingestion of the dicarboxylic acids (C_6 to C_{16}), the homologous acids with 2, 4, or 6 less carbons can be recovered from the urine indicating that the original acids are degraded by beta oxidation. However, direct evidence for omega oxidation of the higher fatty acids is still missing. On this point Verkade stated: "I have been unfortunately as yet to be satisfied with having pointed out the general character of the methyl-group oxidation."

5. Omega oxidation is related to carbohydrate oxidation. In a fasting subject (man) ingesting triundecylin, the observed excretion of dicarboxylic acids

is significantly increased by the subsequent ingestion of glucose. This becomes a central part of Verkade's theory of ketosis. To explain why ingested dicarboxylic acids do not yield ketone bodies during fasting, he looked upon the diminution of diaciduria as a "pathological fact," for when carbohydrate is not available "the classical way of degradation (unilateral beta oxidation) is promoted at the expense of the way of degradation by omega oxidation with subsequent bilateral oxidation."

6. The demonstration of omega oxidation permits it to be stated "nearly with complete certainty" that the food fats are the "parent substances of the C_4 system. Indeed, on degradation of these acids in the manner mentioned, succinic acid appears as an intermediate product."

7. This formation of succinic acid from fat immediately opens a pathway to carbohydrate and Verkade states that there can "no longer be any doubt of the possibility of this process."

It is difficult to evaluate this evidence as presented by Verkade. The fact that certain hypothetical intermediaries are catabolized does not constitute proof that fat metabolism normally goes through these channels, for many substances not remotely related to mammalian metabolism are readily catabolized. Certain weaknesses of the theory are evident: 1. Spontaneous diaciduria (except for traces of oxalic acid) does not occur. 2. Ketosis is difficult to explain since, apparently, ketone bodies do not form from dicarboxylic acids. For example, Edson (57), Mazza (121) and also Califano (29) found no ketone body formation by liver slices in the presence of representative dicarboxylic acids. A subsidiary hypothesis must be postulated that ketosis is a pathological process subsequent to the failure of normal omega oxidation which does not produce ketones. The recent evidence of Shipley (157) that ketone formation in considerable amounts occurs in livers from normal *fed* rats is strongly opposed to this explanation. 3. Finally, omega oxidation requires ipso facto the advocacy of the occurrence of carbohydrate formation from fats. The difficulties of maintaining this position are discussed elsewhere (fats to carbohydrates).

Flaschenträger and his co-workers (64, 65), have reported at length on the subject, and while agreeing with Verkade in some details, differ radically in interpretation of the evidence with respect to the quantitative significance of omega oxidation. In general, Flaschenträger's conclusions are that omega oxidation takes place to a very small degree in the case of the higher fatty acids (less than 1 per cent of total fat oxidation) and that its physiological significance is minimal. Bernhard (12) takes even a stronger view. Incorporating deuterium into dicarboxylic acids, he finds that succinic acid is readily catabolized when fed to dogs or rats, but that adipic, suberic, sebacic, and higher dicarboxylic acids so labeled with deuterium are not metabolized to any appreciable extent. He concluded that omega oxidation is not a step in the normal catabolism of fatty acids.

CATABOLISM OF ODD FATTY ACIDS. The well-established fact that mammalian tissues contain no detectable amounts of odd numbered fatty acids would appear at first sight to deprive them of any physiological interest. On the contrary, all

theories of fatty acid metabolism thus far developed have aspects which only study of the metabolism of odd numbered fatty acids can clarify. Particularly is this so now when theories of ketone formation have had to be re-cast, for the reconciliation of these theories with the experimental evidence on odd fatty acids continues to be difficult.

That the odd fatty acids may be completely oxidized in the body is undoubted as early experiments with glycerol trimargate (i.e., intarvin, a synthetic odd fatty acid mixture) showed when it was fed to diabetics in the pre-insulin days in a misguided attempt to prevent ketosis. However, the chief interest centers around the formation of the ketone bodies from them. Each of the theories of hepatic fatty acid oxidation thus far advanced logically requires that ketones be formed just as readily from the odd as from the even fatty acids. But, as will be seen, this is not the case. According to the successive beta oxidation hypothesis, acetic acid should be formed from odd as from even fatty acids. But acetic acid is well established to be ketogenic (cf. supra). In the light of the early experiments of Embden and Marx (62) showing that odd numbered fatty acids perfused through dogs livers did not yield ketones, Friedman (69) vigorously opposed the hypothesis. He offered no satisfactory alternative. Obviously the beta oxidation-condensation hypothesis is, in this respect, no different from the successive beta oxidation hypothesis. Whatever the hypothetical two carbon unit, whether acetic acid, glyoxylic acid, or some radicle "in statu nascendi", if its mode of formation is the same, it should yield ketone bodies just as readily from even or odd numbered fatty acids. Nor does the multiple alternate oxidation hypothesis escape the dilemma, for it becomes necessary to explain why an enzyme which neatly "fits" an even numbered fatty acid bringing about its rapid degradation to ketone bodies, completely or partially fails to do so in the case of the odd-numbered fatty acids.

Recent work, in one sense, has eased the situation, and, in another, made it worse. The idea current for a long time, based chiefly upon the early work of Embden and Marx (62) was that odd-numbered fatty acids when perfused through dog's liver gave no significant increase of ketone body formation. The difficulty mentioned by MacKay (115) of insufficient concentrations of fatty acids in the perfusing fluids is not in question here, since the authors obtained abundant ketone body formation with even-numbered acids of higher molecular weight than those odd-numbered ones which failed. Deuel, Butts, Hallman and Cutler (46) concluded, from urinary ketone excretion in feeding experiments with rats, that C_3, C_7, C_9 acids were not, and C_5 but slightly ketogenic. On the contrary they found them glucogenic. Krainick (95) injected into 10 human subjects 50 cc. of 0.4M solution of various fatty acids. He studied the ketone blood levels in addition to the ketonuria, thus eliminating MacKay's objection to Deuel's ketonuria studies, and found regularly a typical curve of increased blood levels of the ketone bodies in the case of C_4 to C_{10} acids, but no increase whatever with the odd-numbered acids, C_3–C_{11}, except in the case of C_7 which gave a minimal increase. The whole question has been recently reopened by MacKay and his co-workers (116), who maintain strongly that odd-numbered

fatty acids produce ketone bodies. They reported (118–119) in feeding experiments on rabbits with adequate liver glycogen and no initial ketosis, that ethyl or glycerol esters of fatty acids containing 5, 7, 11 carbons cause ketonemia. Their data do not permit quantitation of the amounts of ketones formed as compared to those resulting from even-numbered fatty acids, but approximately the same levels of blood ketones were found in either case. Jowett and Quastel (87) also found, using liver slices equilibrated with C_5, C_7 and C_9, fatty acids, a formation of ketone bodies. But an examination of these results (table 4) shows a distinctly less amount than with the corresponding even-numbered ones.

In every case the even-numbered fatty acids produced more ketone bodies than the odd-numbered ones in the ratio varying from 2:1 to 5:1.

Califano (29) also found with liver slices that odd-numbered fatty acid produced only small amounts of ketone bodies.

TABLE 4

Data of Jowett and Quastel (87) showing comparative ketone body formation by liver slices equilibrated in vitro with odd- and even-numbered fatty acids

CARBONS IN ACID	AVERAGE KETONE BODY FORMATION $Q_{ac.}$*
None	0.20
2	0.42
3	0.12
4	2.55
5	0.51
6	2.62
7	0.88
8	1.80
9	0.72
10	1.39

* $Q_{ac.}$ = equivalent cmm. as gas per mgm. dry weight per hour. Multiply by 10 = (approx.) uM/wet gm./hr.

An evaluation of this evidence forces a less advanced position than the older one, and it must be concluded that odd-numbered fatty acids are oxidized to ketone bodies in the liver, but to a much less degree than in the case of the even-numbered ones. However, this fact must still be accounted for by any complete hypothesis of fat catabolism. To date no one has satisfactorily done so.

UTILIZATION OF KETONE BODIES. *Liver.* The fact that the liver itself, even in the fed state, (Shipley, 157) produces ketone bodies makes it difficult to determine whether or not it utilizes them. However, in early experiments, Snapper and Grübaum (161) perfused dogs' livers and found no decrease of added acetoacetic or β-hydroxybutyric acid. More recently, Blixenkrone-Moeller (14) has reinvestigated the problem taking into account the prior rate of ketone formation by perfused cat's liver. Upon the addition of β-hydroxybutyrate to the perfusion fluid he found no significant utilization by either glycogen-rich or glycogen-poor livers. On the other hand, Ciaranfi (34) found with liver slices some increase of

CO_2 production upon addition of aceto-acetate. Kühnau (102) reported that added aceto-acetic acid in the presence of liver is decreased with the formation of a series of reaction products, viz: aldol, acetoacetate, acetaldehyde, succinic, fumaric, malic acids and traces of acetic acid.

From the weight of the evidence, it must be concluded that the liver while forming ketone bodies does not utilize them to any significant extent in its own metabolic activities.

Muscles. Abundant evidence has accumulated that ketone bodies are freely used by the normal muscle tissue. Experiments with perfused surviving limbs of experimental animals were done by Griesbach (76), Snapper and Grünbaum (162), and Toenniessen and Brinkman (179). Eviscerated preparations have been used (Friedmann, 67; Chaikoff and Soskin, 33; Dye and Chidsey, 55), and utilization calculated from blood and tissue ketone body levels following injection of acetoacetate or β-hydroxybutyrate. The evidence uniformly points to a complete oxidation of the substrate. Careful measurements by Blixenkrone-Moeller (16), who determined both the ketone body utilization and oxygen consumption of perfused hind limbs of the cat in the resting state and when working (electrical stimulation), gave as the mean of 11 determinations a ratio of 3.6 moles of oxygen to one mole of acetoacetate consumed. This is remarkably close to the theoretical value of 3.5 and proves that the substrate is completely oxidized.

The arterio-venous ketone difference of certain organs has also been used to show ketone body utilization. Goldfarb and Himwich (73) in such studies reported that striated muscle and heart utilized ketones, but that brain did not. Barnes and Drury (49) also demonstrated the utilization of ketones by peripheral tissues by this method.

The calculation of peripheral utilization from the difference of hepatic ketone production and urinary excretion has been successfully employed, the assumption being made that the excess of the former over the latter represents utilization. In normal and diabetic cats Blixenkrone-Moeller (16) showed that the perfused liver produced ketone bodies greatly in excess of the preliminarily determined ketonuria. Likewise Stadie et al. (168) using surviving liver slices to determine hepatic ketone production, obtained the same results. Crandall and his co-workers (40) inserted cannulae into the hepatic veins of dogs and calculated hepatic ketone production from the blood ketone values so obtained using estimated hepatic blood flows.

Peripheral utilization has also been determined by following blood ketone levels after the injection of acetoacetate or β-hydroxybutyric into nephrectomized animals (Nelson, Grayman and Mirsky, 137). When followed by an analysis of the entire carcass this method becomes quite quantitative. Numerous investigators have studied peripheral ketone utilization by the determination of urinary ketone body excretion alone or associated with studies of blood levels. This method has a limited applicability because there is no quantitative relationship between hepatic ketone body production and urinary ketone excretion or blood levels. For example, Stadie et al. (167) found in diabetic cats that there

may be an enormous hepatic ketone body production with no or minimal urinary ketone excretion. Harrison and Long (78) employed a unique method for the determination of ketone utilization by the muscle. From blood level studies they calculated ketone body concentration in cellular water. Even when blood ketones were high following phlorhizin or anterior pituitary injection, they could find no significant amount of ketone in muscle cell water except at high blood concentrations, thus indicating that muscle metabolism of ketones was sufficiently active to maintain them at practically zero level within the cell.

Heart-lung. Using the isolated heart-lung preparation, Barnes, MacKay, Moe and Visscher (10) first conclusively showed that this preparation utilized ketone bodies. Both the lung and the heart are active in this utilization, a considerable fraction of their total metabolism being thus accounted for. More recently, Merlini (124) demonstrated the utilization of acetoacetate and β-hydroxybutyrate by the isolated rabbit heart.

Kidney. Perfusion studies with animals and human kidneys have shown ketone body utilization (Snapper and Grünbaum, 161). Using slices from rat and guinea pig kidneys, Quastel and Wheatley (142) showed that added acetoacetate was oxidized away; they localized this action to the cortex. They also made the interesting finding that the addition of lactate and glucose markedly increased the rate of ketone body utilization. More recently Shipley (157), also using slices of kidney from fasting rats, showed that the rate of decrease of added ketone bodies far exceeded that of any organ studies. He further showed that the kidney gram for gram exceeded the liver in its ability to synthesize carbohydrate from non-carbohydrate sources. The relation, if any, of this to fat metabolism is undetermined.

Brain. The general statement that brain metabolism is solely carbohydrate in character cannot be regarded as proven. Respiratory quotient determinations on both the intact organ, and with brain slices and emulsions, consistently give values of about 0.95 indicating that metabolites other than carbohydrate are being oxidized. Mulder and Crandall (134) were unable to demonstrate ketone body utilization of brain in situ following production of ketosis by fasting. However, they employed the method of arterio-venous differences which is not sufficiently sensitive for this purpose. Shipley (157), by adding acetoacetate to brain preparations in vitro, demonstrated its total oxidation at a rate similar to that observed in muscle. This finding raises interesting questions as to the type of metabolism of the brain in the fasting or diabetic state.

Adrenals. Boda (20) reported the interesting observation that 30 per cent of the ketone content of the adrenal vein blood is removed in its passage through the adrenals of the dog. He calculated that approximately 83 mgm./kgm. body weight/minute are utilized by the adrenals—a large proportion of the total ketone utilization of the animal.

Phlorhizin poisoning. Abundant ketone body utilization during acidosis induced by phlorhizin has been shown by Barnes, Drury, Greeley and Wick (8), using the method of arterio-venous differences. Harrison and Long (78) also showed the same thing from their calculation of ketones in muscle cell water.

Exercise. The interesting problem as to whether the increased demands of working muscle can be furnished by ketone body utilization was answered unequivocally by Blixenkrone-Moeller (16). In his perfusion studies on isolated surviving hind limbs of cats, he brought about a 10-fold increase in ketone body utilization by causing the muscle to exercise (electrical stimulation). When rats in a state of fasting ketosis were made to work heavily, Drury, Wick and MacKay (54) found a sharp fall in blood ketone levels followed 2 or 3 hours later by a rise. They concluded that exercise first causes an increased ketone utilization by the muscle; after a lag period there is an increased production by the liver to meet the extra demand. The work of Mirsky and Broh-Kahn (128), showing that increased metabolism due to dinitrophenol is accompanied by increased peripheral ketone body utilization is corroborative evidence on this point. Neufeld and Ross (136) also found evidence in exercising man that ketone body utilization is increased.

Diabetes. For a period of forty years the convergence of two theories concerning fat metabolism dominated the question of the utilization of the ketone bodies by the diabetic organism and popularized the application of aphorisms to the solution of the problem. These theories dovetailed neatly one into the other. The theory of successive beta oxidation explained how fatty acids were degraded two carbon atoms at a time leaving a ketone residue; the two carbon unit split off (acetic acid by presumption) sufficed in the main for the metabolic needs of the complete diabetic. The theory of obligatory coupling required that ketone oxidation be coupled with that of carbohydrate. The combined theories explained the ebb and flow of the ketosis of the diabetic. Imagination likened this to the "flame of the carbohydrate" which completely consumes fat, or the faulty engine which "smokes" with ketones when diabetes mal-adjusts the carburetor. A third theory, different in nature, supposed that, owing to insulin lack, fatty acids are abnormally converted at a prodigious rate into glucose as well as ketone bodies both of which are excreted because their rate of production by the liver is greatly in excess of the ability of the peripheral tissues to utilize them. Chaikoff and Soskin (33) first conclusively showed that the diabetic dog burned ketone bodies as readily as did the normal animal. They injected acetoacetate into the eviscerated, depancreatized animal and studied the rate of disappearance. These experiments were repeated and confirmed (Dye and Chidsey, 55; Friedmann, 67; Mirsky and Broh-Kahn, 127). Blixenkrone-Moeller (16) showed in a convincing, manner in perfusion experiments on the hind limbs of diabetic cats an active utilization of ketone bodies which was increased 3 to 5 fold during exercise. The oxygen consumption measured during rest and work indicated that the ketone bodies were completely oxidized. He also showed, by a comparison of the rate of ketone body formation from perfused livers of diabetic cats and the preliminarily determined urinary ketone body excretion, that there was a great excess of ketone formed which was undoubtedly burned in the periphery. Stadie (165, 166) reviewed the subject and showed in the following ways that the diabetic animal utilized ketone bodies freely despite the complete absence of insulin, and with no relation to the oxidation of carbohydrate: 1, the ketone

production by liver slices from diabetic cats was greatly in excess of the ketone excretion in the urine determined in a preliminary period: the excess represented ketone utilization; 2, muscle tissue from diabetic cats equilibrated with acetoacetate oxidized it away at a rate sufficient to take care of a considerable fraction of the total energy requirements; 3, ketone bodies produced by liver slices from diabetic cats were oxidized away when the slices were simultaneously equilibrated with muscle mince from the same animal; 4, hepatic ketone production measured by portal-hepatic ketone differences in diabetic cats was again greatly in excess of urinary ketone excretion; 5, by an analysis of the best metabolic data in the literature on human cases of diabetes treated before the days of insulin when ketosis was a common occurrence, Stadie concluded that the diabetic could completely catabolize about 2.5 grams of fat per kgm. body weight per day without ketonuria. Since a considerable part of this total fat catabolism went by way of preliminary partial oxidation to ketone bodies in the liver, it was clear that the human diabetic could oxidize ketone bodies freely, independently of insulin or carbohydrate oxidation. A summary of these data is given in the accompanying table 5.

THE MODE OF OXIDATION OF FAT IN MUSCLES. The problem of what metabolites are completely oxidized to supply energy for the muscles in rest and exercise has been of major interest to physiologists for many years. The older idea that carbohydrate is the sole muscle fuel had to be abandoned in view of the conclusive evidence that muscles oxidize the ketone bodies freely. The question as to whether or not fatty acids per se could be oxidized directly has been a subject of controversy, and Gemmill (71) in his recent review of the fuel for muscular exercise concluded that "There is no experimental evidence at the present time for the direct utilization of fat by mammalian muscle." The present reviewer cannot agree with this position. In an evaluation of the evidence (165) he proposed that the total fat catabolism could be represented by:

a. *Indirect oxidation*, i.e., a preliminary partial oxidation of fatty acids in the liver to ketone bodies followed by complete oxidation of the ketones in the periphery (chiefly muscle), and (*b*,) *direct oxidation*, initiated and completed in the periphery. He found (165) with liver slices from depancreatized cats, ketone body formation equivalent to 0.09 gram of fat per kgm. of body weight per hour whereas the total metabolism of the diabetic cat was of the order of 0.32 gm./kgm./hr., i.e., only about 30 per cent of the total metabolism could be accounted for by indirect fat utilization. The balance must have been by direct oxidation, unless it was assumed that the diabetic cat could utilize glucose. These values for ketone utilization were in substantial agreement with those of Blixenkrone-Moeller (14) using perfused livers from diabetic cats. Crandall, Ivy and Ehni (40) came to a similar conclusion when they estimated the hepatic ketone production in angiostomized dogs. After five days of fasting, when all of the available carbohydrate stores were used up, they estimated that only about 50 per cent of the total energy requirements of the animals could have been met by the ketone bodies formed in the liver. The balance must have been by direct oxidation of fats in the muscle.

Another difficulty when the possibility of fat oxidation by the muscles is excluded has been pointed out by Stadie (165). If the entire muscular metabolism during exercise in the fasting state when carbohydrate stores are depleted, is dependent upon indirect fat oxidation, the liver will have to increase its oxygen

TABLE 5

Calculation from data in the literature of ketone body utilization by the peripheral tissues of the normal and diabetic animal

	MEAN KETONE UTILIZATION PER KGM PER DAY
	gram
Chaikoff and Soskin (33)	
By measurement of rate of disappearance of injected ketone bodies from blood of:	
Diabetic eviscerated dogs	2.9 ±0.1
Normal eviscerated dogs	3.4 ±0.6
Friedmann (67)	
By measurement of ketone body excretion when injected with very large amounts of acetoacetate	12 (maximum)*
Blixenkrone-Moeller (16)	
By comparison of ketone formation of perfused diabetic cat livers with prior ketone excretion	3.9 ±0.3
By measurement of rate of disappearance of ketone bodies from blood perfused through:	
Resting hind limbs of normal and diabetic cats	2.7 ±0.4
Working hind limbs of normal and diabetic cats	13.0 ±0.7*
Dye and Chidsey (55)	
Injection of acetoacetate at high rate into nephrectomized-depancreated dogs (maximum)	18.*
Stadie, Zapp and Lukens (167)	
1. By comparison of ketone formation by diabetic cat liver slices with prior urinary ketone body excretion	2.7 ±0.4
2. By measurement of ketone body utilization by diabetic cat muscle mince in presence of added acetoacetate	2.2 ±0.6
3. By measurement of ketone body utilization of diabetic cat muscle mince simultaneously equilibrated with diabetic cat liver slices	5.0 ±1.0
4. By measurement of the portal-hepatic ketone difference in diabetic cats	3.0 ± 1.
Mean basal ketone utilization	3.0 ±0.3
Equivalent grams of fat	2.3

* Excluded from basal mean.

uptake very greatly to form the necessary ketones. In a hypothetical case the hepatic oxygen uptake during moderate exercise was calculated to be approximately 1000 micromoles per gram per hour, ten times that determined for the basal state (Bancroft and Shore, 5). It is difficult to suppose that the oxygen uptake in the liver ever attains such high levels.

Crandall and his co-workers (37, 40), by studies of hepatic ketone production in the angiostomized liver and by blood ketone studies, describes a marked lag in hepatic ketone production upon fasting in dogs and also man. Only after 2 to 3 days does hepatic ketone production reach maximum levels, and even then it is only sufficient to account for about half of the total metabolism. In the face of this lag and the loss of available carbohydrate stores, it is hard to explain how the metabolic requirement can be met without supposing direct fat oxidation in the muscles.

Bearing on the question are the experiments of Barker (6) who found no increase of ketonuria or protein metabolism following exercise in diabetic dogs. To supply these extra calories one would have to suppose an instantaneous and large increased production of ketones to extraordinary heights by the liver, or a marked increased carbohydrate metabolism, or direct fat oxidation. The evidence is very much against the first two alternatives.

Nor is more direct evidence lacking. Barnes, Drury, Greeley and Wick (8) measured the peripheral ketone utilization and oxygen consumption by arteriovenous differences in phlorhizinized dogs. They could only account for approximately half of the metabolism by ketone utilization. In studies on heart-lung preparations (10) accompanied by β-hydroxybutyrate injections they calculated that the heart could oxidize fats directly to the extent of $\frac{2}{3}$ of its metabolic requirements.

Studies of the respiratory quotient of muscles in the fasting state either in vitro (138) or in the intact state (81) give values close to the theoretical value of 0.7 for oxidation of fat. Cruickshank (43, 44), who spent years perfecting his ability to handle the heart-lung preparation for metabolic studies, found repeatedly in diabetic hearts R.Q. values of 0.7 which promptly rose to 1 following the injection of insulin. Those who are skeptical of the significance of R.Q. values in isolated tissues must explain, if they exclude direct fat oxidation, why diaphragms from fasting rats when equilibrated without glucose almost invariably show R.Q. values close to 0.7. Drury and McMaster (53) contributed valuable evidence by determining the R.Q. of rabbits deprived of their livers. Following fat feeding the R.Q. was not significantly changed from the preliminary period before hepatectomy. Their average R.Q. value in the preliminary period was 0.73 ± 0.01, and remained unchanged even as late as 24 hours after the operation. Apparently, the organism deprived of liver is still capable of burning fat independent of any possible formation of carbohydrates or ketones by prior action of the liver. In other words, the low R.Q. is a true fat combustion R.Q. and not the algebraic sum of processes going on in the liver and muscles simultaneously. Gemmill's own work (70) in which he determined the fat and carbohydrate content of phlorhizinized rat muscle in the resting and exercising state cannot be used as proof that muscle is unable to oxidize fat. He found a decrease of carbohydrate but none of fat. However, his data do not include analyses of blood which must be considered as a possible source of fat. Furthermore, phlorhizinized muscle is still capable of oxidizing glucose; hence his preparations might not have been suitable ones for the demonstration of direct fat combustion. But even if the sum of this evidence was insufficient, the direct evidence of

Cruickshank and Kosterlitz (42) remains. In many experiments on dogs' hearts, they measured the total initial and final phospholipids, fatty acids, and cholesterol. When there was low cardiac glycogen to favor fat oxidation, they were able to demonstrate beyond doubt that the heart can and does utilize stored fatty acids per se for its metabolic needs. This confirms the older observations of Visscher (181) who, on the basis of oxygen and carbohydrate balance studies, had made the same conclusion.

THE INTEGRATION OF CARBOHYDRATE AND FAT METABOLISM. *Ketolysis, antiketogenesis, and the ketogenic-antiketogenic ratio.* It has long been known that there is a close integration of carbohydrate and fat metabolism, the mechanism of which, despite a multitude of experimental papers and hypotheses, still remains obscure. In fasting or in diabetes mellitus carbohydrate oxidation is diminished or absent; hence fat becomes the major metabolic ingredient. Upon restoration of carbohydrate oxidation, either by the ingestion of it, in fasting, or the injection of insulin, in diabetes, fat metabolism recedes into the background, and the metabolic mixture becomes predominantly of a carbohydrate character. The explanation of this phenomenon has stimulated the advocacy of contending hypotheses only one of which can safely be said to be eliminated from consideration.

The obligatory coupling of carbohydrate-fat oxidation: ketogenic-antiketogenic ratio. This theory, which goes back as far as Gaelmuyden and was in reality a complement of the successive beta oxidation hypothesis, explained the recession of ketonuria following resumption of carbohydrate oxidation in diabetes mellitus and fasting. It supposed that acetic acid or other two carbon compound split off by hepatic beta oxidation of fatty acids, could be freely used in the periphery, but that the ketone residue could not unless its oxidation was directly coupled with the oxidation of carbohydrate or some "antiketogenic" intermediate, derived therefrom, viz:

$$\text{Ketone} + \text{antiketone} + O_2 = CO_2 + H_2O$$

The assumptions were clearly stated by Shaffer (148): "Antiketogenesis in the human subject is based upon a ketolytic reaction in the body between acetoacetic acid, the first formed of the acetone bodies, and a derivative of glucose (or other antiketogenic substance), the compound being further oxidized, but that failing to react with ketolytic substances, acetoacetic acid is resistant to oxidation, accumulates, and . . . is excreted. The fact that one finds at the threshold of ketosis an approximately constant ratio between the number of molecules of the precursors of acetoacetic acid and of glucose in the metabolic mixture, must mean that the further oxidation of acetoacetic acid constantly taking place under normal conditions is accomplished through a chemical reaction with a derivative of glucose. . . ."

The stoichiometrical ratio between the ketones and antiketones was termed the ketogenic-antiketogenic ratio and was usually stated to be 2:1. Innumerable attempts were made to measure this ratio experimentally in man and animals. Stadie (165) re-examined the best data on human diabetes available

in the literature. The 12 cases were all classical ones, reported before the advent of insulin when marked ketosis was, of necessity, a frequent accompaniment of the disease. The data include calorimetric measurements of the metabolic mixture of proteins, fats, and carbohydrates together with the total ketone excretion. Hence it was possible to calculate the total carbohydrate or so-called antiketones oxidized. In addition, from the total fat catabolized and the urinary ketone excretion, the total fat utilized or its equivalents in ketones could be calculated. His analysis showed that there was no significant relation between the fat utilized and the "antiketones" oxidized, i.e., the ketogenic-antiketogenic ratio had no reality. On the other hand, there were strong indications that these patients, many of whom were almost complete diabetics, were oxidizing ketones or fats without simultaneous oxidation of carbohydrates, i.e., obligatory coupling was unnecessary. Stadie stated a simple hypothesis of fat metabolism in diabetes mellitus which would conform to the observations in these clinical cases and with experiments with animals, viz.: up to a certain level all fat catabolized is completely oxidized; hence there is no ketonuria. Beyond this level (approximately 2.5 gm./kgm./day) all fat catabolized is not completely oxidized; hence part of the fat catabolized is excreted unburned in the form of ketone bodies. The action of carbohydrate is simply to spare fat oxidation; there is no molecular reaction, hence there is no molecular ratio between the two.

The work of others who have measured directly in experimental animals the simultaneous utilization of ketone bodies and carbohydrates when intravenously injected, has shown that the rate of utilization of one is not affected by that of the other (130, 67). In particular, Dye and Chidsey (55) determined total ketone body and carbohydrate utilization in nephrectomized-normal and nephrectomized-depancreatized dogs following the intra-venous injection of acetoacetate and glucose in varying amounts. From their data they found the ratio between these two utilizations to vary from 0.7 to 19.6. Furthermore, the rate of utilization of ketone bodies was independent of the blood sugar level, carbohydrate utilization, evisceration, or pancreatectomy, being solely a function of the concentration of blood ketones.

Waters, Fletcher and Mirsky (184) came to equally decisive conclusions. They studied carbohydrate and ketone utilization in the dog heart-lung preparation and found no positive correlation between the two. Their "ketogenic-antiketogenic ratios" varied from 0.2 to ∞.

The ketogenic-antiketogenic ratio has proved to be an ignis fatuus. It is the belief of the reviewer that there is no evidence for the obligatory coupling of ketone-carbohydrate oxidation and that a strictly stoichiometrical, small whole number ratio between ketogenic and antiketogenic substances is non-existent.

Ketolysis. On the other hand, the ideas behind the theory of ketolysis cannot be dismissed lightly, particularly in view of recent work indicating a possible integration of fat and carbohydrate metabolism involving citric acid and the four carbon dicarboxylic acids (v. infra: Wieland and Rosenthal, 191; and Breusch, 22). A careful distinction should be made between "ketolysis" and "obligatory

coupling." The latter implies a strict simple molecular ratio between two substances when reacting; without the reaction no oxidation whatever of ketones occurs. The former is not limited to this, but might include the case in which fat metabolism already occurring independently might be *accelerated* by interaction with carbohydrate intermediaries. It is conceivable that by mechanisms still undefined carbohydrate would bring about further oxidation of ketone bodies or even fatty acids in unlimited molecular ratio. In other words carbohydrate might act "catalytically" in promoting the oxidation of fat or its intermediaries.

For example, Shaffer (148) demonstrated in model experiments in vitro that under certain circumstances glucose catalysed the oxidation of acetoacetate. Shapiro (150) found in feeding experiments with rats that only glucose formers were effective in diminishing the ketonuria following acetoacetate injection.

The same results have been obtained with amino acids (Butts, Dunn and Hallman, 28; Butts, Blunden and Dunn, 25). Deuel (48, 49) has engaged in a sharp polemic with Mirsky (129, 130) on this subject and has stuck staunchly to the position that the phenomenon of ketolysis is real. He criticized the opposing experiments of Mirsky on technical grounds and cites his own work in support. He found in rats a greater rate of disappearance of injected 1-β-hydroxybutyrate when glucose was simultaneously given. Deuel also studied the endogenous ketonuria in rats on prior high fat diet, and following administration of butyric acid. Glucose—but not an isodynamic amount of alcohol—significantly diminished ketonuria in both cases. More significant is his calculation that in the endogenous ketonuria of fasting, the administration of a small amount of glucose iso-dynamically equivalent to approximately 2 per cent of the fat catabolism resulted in the excretion of an average of 3.2 mgm. per 100 gm./d. compared to 43.2 gm./100 gm./d. in the controls. Bobbitt and Deuel's (19) experiments with liver slices in the presence of butyrate without or with added glycogen must be viewed with more caution particularly since they are somewhat opposed to those of Edson (58). They concluded from their data that in the presence of glycogen more butyrate disappeared and less ketone bodies were formed indicating a possible "catalytic removal of ketone bodies by carbohydrate, either by oxidation, or by formation of compounds which do not react with Deniges reagent." But their calculated butyrate decrease was the small difference between two large numbers, and in their balance they did not consider the possibility that butyrate might be converted to glucose (Blixenkrone-Moeller; 15; Buchanan, Hastings and Nesbitt, 23).

In view of this evidence and the recent developments in intermediary fat metabolism in the periphery, the reviewer feels that it would not be wise to exclude a possible effect of carbohydrate catabolism upon that of fat by some mechanism which could be described by the term "ketolysis" in some more far-reaching sense than the usual one.

Substrate competition. In discussion of the integration of fat and carbohydrate metabolism, substrate competition plays a rôle. Edson (58) discussed it at some length. The supposition is that tissues oxidize one substrate in preference to

another; there is no significant change in total oxygen uptake since this is limited by relatively non-specific mechanisms. For example, the deamination of l-amino acids by surviving kidney slices is diminished in the presence of a readily oxidizable substrate such as lactate or pyruvate. Quastel and Wheatley (141) found that propionic acid, but not glucose or lactic acid, inhibited the formation of acetoacetic acid from butyric acid by liver slices. Edson (58) found, using liver slices, that spontaneous ketone formation can be significantly altered by the addition of certain substrates without, as expected from the theory, there being any great change in total oxygen uptake. Glycerol, sorbitol, glyceraldehyde were strongly antiketogenic; whereas glucose, fructose, mannose, galactose were moderately so, and glycogen not at all. This antiketogenic effect is a simple "sparing" of fat and does not involve any direct chemical interactions.

Stadie et al. (167) studied the effect of fructose in diminishing the ketone formation of liver slices from diabetic cats. Their results are summarized in table 6.

TABLE 6

Average decrease (per cent) from control of ketone formation by liver slices from diabetic cats. Micromole per gram per 2 hours (Stadie, Zapp and Lukens, 167)

INSULIN	FRUCTOSE	FRUCTOSE + INSULIN	FRUCTOSE + FUMARATE	FRUCTOSE + FUMARATE + INSULIN	FUMARATE	FUMARATE + INSULIN
+9	−12	−23	−20	−42	−26	−26

The decrease effected by fructose was always enhanced by the presence of insulin, but the greatest effect was found with fructose, fumarate and insulin. They also found that the effect of fructose and insulin was enhanced if the slices were equilibrated for a longer period of time (4 hrs.). These results, while indicating a possible substrate competition by fructose enhanced by insulin, also indicate involvement of the four carbon di-carboxylic acids in fat catabolism as originally suggested by Szent-Gyorgyi (176).

Hepatic glycogen and ketone formation. The inverse relation between the glycogen content of the liver and its rate of ketone formation has long been known. Blixenkrone-Moeller (14) in his discussion of this topic pointed out the following relations: 1, in general hepatic ketone production tends to be higher in livers with the greatest fat content; 2, more significant is the glycogen content: in normal animals fasting brings this level down to about 1 per cent and there is moderate ketone formation; in depancreatized animals having hepatic glycogen levels less than 1 per cent, ketone production is highest irrespective of the level of liver fat; 3, when in normal animals liver glycogen is brought below 1 per cent by the using of adrenalin or phlorhizin, ketone production is much higher, but even then does not consistently reach the levels attained by diabetic livers. Blixenkrone-Moeller eliminated as a possible explanation for this inverse correlation of glycogen and ketone formation, "sparing" of fatty acid oxidation by glycogen. For upon correction of oxygen uptake for ketone formation, he finds

no difference in the R.Q. of glycogen-rich or glycogen-poor livers. Stadie et al. (167), found essentially the same thing. Corrected in the same way the R.Q. of liver slices from diabetic cats was not significantly different from that of normal cats, (0.81 ± 0.05). When hepatic ketone formation was markedly reduced by adequate treatment with insulin in a preliminary period, the non-ketone R.Q. of liver slices was lowered rather than raised.

On the other hand, Mirsky (129) and his co-workers studied ketonuria in diabetic dogs before and after massive doses of glucose intravenously and came to opposite conclusions. They stated that their "data are in accord with our working hypothesis, that the production of ketosis by the liver itself is always due to a decrease in the amount of glycogen available for utilization by the liver itself . . . and that glycogen formation is an obligatory step in the oxidation of carbohydrate and the prevention of ketosis. . . . The production of ketone bodies is attributed to a compensatory acceleration in the metabolism of fatty acids and protein in the liver in consequence of a decrease in the amount of carbohydrate available for oxidation by the liver itself." The reviewer knows of no evidence requiring the formation of glycogen from glucose prior to oxidation. In fact, all schema of glycolysis place hexose monophosphate as a common intermediate step between glucose, glycogen and the products of glycolysis. Hence, if this type of substrate competition explains the suppression of hepatic ketosis, glucose itself, which is abundant in the blood in diabetes, should be just as efficient an antiketogenic agent, which it is not. Other facts must also be considered. 1. The shorter fatty acids when administered give rise to abundant ketone formation even in the presence of abundant readily available glycogen in the liver (116); 2, there is a lag in maximum ketone production in certain species on fasting (v. supra); i.e., maximal ketone production is reached long after hepatic glycogen has reached its lowest levels; 3, ketone production continues after liver glycogen has been raised by feeding (Wick and MacKay, 189); 4, hepatic ketone production in Houssay cats is significantly lower than that of normals despite the almost complete absence (0.03 per cent) of glycogen in the liver (Stadie et al, *167*).

In a purely speculative paper Witzeman (193) reviewed the evidence and formulated a hypothesis bearing on the problem of reciprocal integration of carbohydrate and fat catabolism. He summarizes the pertinent evidence in 20 categories. Unfortunately much of recent evidence is ignored, and in instances questions which are still in doubt are categorically asserted to be proven. In other instances, evidence cited has been controverted by more recent work. Nevertheless his discussion of fat catabolism is interesting, but the finer details of it cannot be considered here. According to Witzeman, fat metabolism embraces the following events: 1, transportation of fats by conversion to hydrophylic phospholipids and cholic acid-cholesterol complexes; 2, combination of these hydrophylic compounds with oxidizing enzyme systems concerned with fat catabolism; 3, oxidative mechanisms bringing about the two typical end results of fatty acid catabolism: *a*, complete combustion; *b*, production of ketone; 4, "recapture" of soluble fatty acid intermediaries (acetic acid, aceto-acetic

acid, etc.) and oxidation by enzyme systems primarily involved in carbohydrate metabolism. Such a system, presumably by mass action effects, might integrate carbohydrate and fat metabolism. Although speculative and dogmatic, Witzeman's paper repays study. At least one can echo his hope "that the general catalytic channels have been worked out in amazing detail in the carbohydrate physiology . . . we can scarcely expect anything less satisfactory for fat, when the story is told."

The integration of carbohydrate-fat metabolism must await further experimentation. Substrate competition, endocrine influences, cyclical involvement of the four carbon dicarboxylic acids, phosphorylations, are some of the factors which must be involved in the complete theory.

FAT TO CARBOHYDRATE. This question continues to be a cause celebre among biochemists, but certain new lines of thought have arisen which may reconcile old conflicts. The liver was hitherto believed to be the sole source of blood sugar, but the kidney has now advanced to the front rank as a glucose former. Information about its importance in this rôle is still meagre. The evidence that the diabetic or phlorhizinized liver does not convert fatty acids to carbohydrate is very strong, but no such dogmatism can be maintained with respect to the normal liver. Involvement of the four carbon dicarboxylic acids in the peripheral oxidation of fatty acids is being actively discussed. Perhaps this foreshadows a transformation of fatty acids to carbohydrate intermediaries which keeps pace parri passu with oxidation, so that outpouring of glucose into the blood from peripheral sites never occurs. The use of isotopic carbon permits the establishment of definitive pathways of metabolism; categorical answers to multitudinous questions concerning fat metabolism may be found by this means.

There are several reviews devoted solely to the subject (131, 165). The evidence pro and con can be grouped under the following heads:

1. Type of fuel for muscular work.
2. Total non-protein R.Q. in rest and exercise.
3. D:N ratios: Normal, diabetic, adrenalized, or phlorhizinized animals, organs, or isolated tissues.
4. Hepatic oxygen: ketone ratio.
5. Intermediate pathway of fatty acid derivatives.

Fuel for muscular work. Many early workers assumed that muscle utilized carbohydrates only, hence a conversion of fat to glucose by the liver was obligatory. In exercising subjects the loss of muscular efficiency in subjects fasting or on fat diets was assumed to be due to the loss attending this conversion. The possibility which we now know to be real that the conversion was to ketones was never considered (Krogh and Lindhard, 101). The evidence for both direct and indirect oxidation of fat by muscle has been reviewed here and carbohydrates can no longer be regarded as the sole fuel of muscle.

Total non-protein respiratory quotient. The total R.Q. is the summation of oxidative processes throughout the body. Assume a fasting subject with depleted carbohydrate stores to be converting fat to glucose in the liver. His

non-protein R.Q. will be that of pure fat oxidation (0.7) unless: *a*, there is a loss of glucose or ketone bodies in the urine, as in diabetes or phlorhizin poisoning or, *b*, there is storage of carbohydrate as glycogen in muscles of liver, or of CO_2 in the body buffers.

An evaluation of the clinical and experimental data in diabetes fails to reveal well authenticated examples of non-protein R.Q. significantly below the theoretical 0.7 for complete fat combustion. Hawley (79) has reported a searching investigation on this subject using phlorhizinized dogs and determining the R.Q. and the D:N ratio. She was unable to find any evidence indicating the conversion of fat to carbohydrate. In humans under exercise Stewart, Gaddie and Dunlop (173) came to the same conclusion from data on the R.Q. Soskin (164), discussing the subject, gave weight to the small decreases of non-protein R.Q. below 0.7 reported in the literature as supporting the hypothesis of fat to carbohydrate. But granting that these small differences are real, they would indicate only a small conversion of fat to glucose, i.e., about 10 to 20 per cent of the total fat catabolized. In the average diabetic or phlorhizinized animal the formation and the excretion of 50–100 grams of glucose from fat would result in a marked decrease in the non-protein R.Q. well below 0.7 and outside of the range of possible experimental error.

Werthessen (188) trained rats to eat but once a day and measured the R.Q. at frequent intervals. In most instances the R.Q. was equal to or greater than 0.7 but there were occasional sharp brief changes to 1.7 or 0.3. The author concluded that these changes indicate a breakdown of total metabolism into steps. Without confirmation of these unusual and interesting findings with data on the CO_2 and ketone storage or elimination, these findings cannot be evaluated in relation to the present problem.

D:N ratio. The situation here is the same as with the R.Q. Any considerable fraction of total fatty acid catabolism resulting in glucose formation would make the D:N ratio undisputably high. In ignorance of the exact proportion of protein convertible to carbohydrate under varying conditions, it is impossible to deny or assert from D:N data available that a *small* fraction of the catabolized fat may be converted to glucose. The occurrence of well-authenticated D:N ratio greater than 4 is rare. Soskin (164) found in depancreatized dogs D:N ratios over 3.2 in only about 10 per cent of the observations. Chaikoff and Weber (31) found high D:N ratios in dogs injected with adrenaline and concluded therefrom that carbohydrate was derived from fat, but Bollman, Mann and Wilhemj (21) showed clearly that the extra glucose came from the muscle glycogen, a result confirmed by Bachrach, Bradley and Ivy.

Liver D:N ratios. Here the evidence is equivocal. Burn and Marks (24), using perfused livers of normal fat fed and one diabetic dog, found extra amounts of glucose synthesis which they concluded came from fatty acids. However, Gregg (75) repeating these experiments failed to get consistent results and asserted that the distribution of residual glycogen was so variable that the use of perfused liver was a highly unsatisfactory method of studying the question. Blixenkrone-Moeller (14) concluded that ketone formation from fatty acids

was the dominant reaction in diabetic cat livers. He nevertheless considered a small conversion to glucose possible. Heller (80) determined the hepatoportal differences of sugar and lactic acid in intact 3-day fasted normal dogs and compared them with the urinary nitrogen excretion. In comparison with the urinary nitrogen, he calculated an excess of sugar of 2.2 mgm./gm.liver/hour, even on the assumption that all of the protein and lactic acid is converted into glucose. Conclusions involving an estimated hepatic blood flow, as does this one, have doubtful value, particularly in this case. For if Heller's estimation is too high by 20 to 30 per cent the calculated extra glucose vanishes. Jost (86) also perfused normal and diabetic livers with phospholipids (lecithin and cephalin). He observed a large increase of glucose and glycogen formation. These he attributed to conversion of phospholipid to carbohydrate. However, in depancreatinized dogs, he was completely unable to demonstrate any extra formation of carbohydrate. This he explains away by stating: "The transformation of ketones to carbohydrates needs the intervention of some intermediary metabolic product of carbohydrate oxidation." (Reviewer's translation.)

Hepatic oxygen-ketone ratios. In the diabetic liver, as contrasted with the normal, the metabolism is mainly that of fat oxidation. Hence the oxygen uptake, particularly if it can be corrected for other known oxidations, will give a *maximum* value for fatty acid oxidation. Since both ketone and glucose formation require oxygen, the O_2:ketone ratio under these circumstances becomes a clear indicator of the course of the metabolism. Blixenkrone-Moeller (14) measured this ratio in perfused diabetic cat livers. He concluded that the main product of fatty acid oxidation was ketone bodies, but he did not exclude the possible formation of small amounts of glucose. The contention of Gemmil and Holmes (72) that the low R.Q. values observed with liver slices from fasting or fat fed rats is evidence for the formation of glucose from fatty acids cannot be accepted, since ketones formation was not measured.

Stadie, Zapp and Lukens (167) measured the hepatic oxidative metabolism of liver slices from diabetic cats. The sum of oxygen required for ketone and CO_2 formation together with that required for oxidative deamination as determined from urea and ammonia formation, was not significantly different from the total observed oxygen uptake. In other words there was no oxygen available for glucose formation from fatty acids and the conclusion appeared inescapable that the diabetic liver oxidizes fatty acids to ketones only and no glucose whatever is formed in the process.

Intermediary pathways of fatty acid derivatives. Attempts to demonstrate the conversion of acetoacetate to carbohydrate have universally failed. Deuel, Butts, Hallman and Cutler (46) were unable to demonstrate any significant hepatic glycogen deposition in rats fed acetoacetate, butyrate, caproate, caprylate, or oleic acid, although there was abundant formation following valeric, heptylic or nonylic acids. Further, they could find no evidence for transformation of acetic acid to glucose in phlorhizinized dogs (50); Stöhr (174) obtained the same results. Blixenkrone-Moeller (14) although convinced that the major portion of hepatic fat oxidation in diabetic cat livers resulted in ketone body

formation, still asserted the possibility of a small amount of glucose formation from fats. However, he could find no extra glucose formation when acetoacetate was perfused through livers. Stadie, Zapp and Lukens (168) re-examining the claim of Weil-Malherbe (185) that the kidney converted acetoacetate to glucose, could find no evidence for it. Furthermore, in balance studies the decrease of fatty acids in liver slices was fully accounted for by ketone body formation (169). Using liver slices which they showed could synthesize carbohydrate from butyric acid, Cross and Holmes (41) could show no carbohydrate formation from acetic acid. Haarman and Schroeder (77) claimed that liver slices convert di-hydroxy butyric acid to carbohydrate. Upon analysis, their data show an apparent synthesis under anaerobic as well as aerobic condition, an impossible result. Failure to remove acetone completely before lactic acid analysis may be the cause of this discrepancy.

Butyrate appears definitely to be a precursor of glucose in the liver as has been shown by Blixenkrone-Moeller (15) in perfusion experiments, and more recently by Buchanan, Hastings and Nesbitt (23) using radio-active carbon in the carboxyl group. Hitherto, evidence for this reaction was lacking (Deuel, Butts, Hallman and Cutler, 46). Blixenkrone-Moeller (15) found no formation of glucose from valeric acid when it was perfused through normal and diabetic livers.

Alternative pathways of hepatic fatty acid oxidation. It is conceivable that in some circumstances, e.g., in diabetes, the liver oxidizes fatty acids to ketone bodies, while in others, e.g., in the normal state, the conversion is to carbohydrate. In other words, in the general reaction: Fatty acid + oxygen = n ketone + (1-n) glucose, n may vary from 0 to 1. This conception might reconcile conflicting data though the evidence for it is hardly more than vaguely suggestive: 1. The constant finding in diabetic or phlorhizinized animals of D:N ratios (ca. 3.5) supporting the no carbohydrate hypothesis would be reconciled with the high (20 or more) ratios found in perfused normal livers (Burns and Marks, 24; Heller, 80). 2. Jost's (86, v. supra) failure to find high D:N ratios in perfused diabetic in contrast to his findings with normal livers would be comprehensible. 3. Verkade's (186) inference that "ketone formation in the diabetic represents an abnormal departure from normal omega oxidation" would have more definite meaning. 4. The lag, often marked, of ketone formation in fasting animals, might mean to those who deny direct fat oxidation in the muscle that in the initial period of the fast the fuel for muscle energy is glucose from fat. Later the type of hepatic metabolism would change and ketones would be the main product of fatty acid oxidation. 5. The observation by Lehninger (108) that in fasting, acetopyruvate is ketogenic, whereas in the hypoglycemia following insulin injection, it appears to be glucogenic indicates that one substance may follow two different metabolic pathways under two different metabolic conditions.

Summary. The reviewer concludes from evaluation of this evidence that fatty acids are not converted to glucose by the liver of the diabetic or phlorhizinized animal. The evidence that the reaction occurs in normal livers is meagre and indirect; nevertheless judgment must be suspended. No conclusive evidence

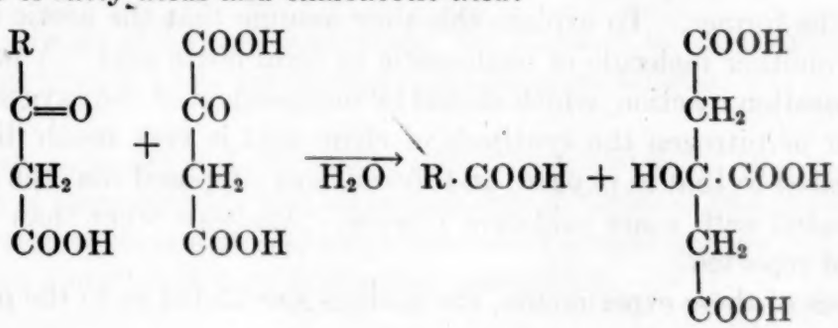
for the conversion in the kidney is at hand. The possibility that the muscles may form carbohydrate intermediaries during fat oxidation must be considered, but data in this field are still controversial. There is no evidence that such hypothetical carbohydrate intermediaries are precursors of blood glucose. If formed, they must be further oxidized *in situ*.

INTERMEDIARY PATHWAYS OF FATTY ACID DERIVATIVES. Edson and Leloir (59) studied the mechanism of ketone body metabolism in various tissues using slices. They found that pyruvate and fructose accelerated the anaerobic disappearance of acetoacetate in the liver but had no marked influence in other tissues except pigeon kidney. The reaction: acetoacetate → β-hydroxybutyrate appears to be demonstrable in most tissues. With kidney tissues, acid accumulation suggested that acetoacetate breaks down through acetic acid (cf. Lehninger) and malonate inhibition suggested that the latter is oxidized by some such scheme as:

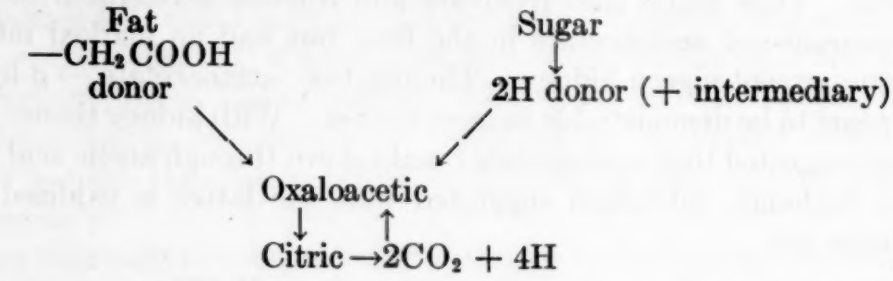
$$\begin{array}{lcl} \text{Acetic acid} & & \\ + & \xrightarrow{-2H} \alpha\text{-ketoglutaric acid} \xrightarrow[-CO_2]{H_2O,\ -4H} \text{Fumaric acid} \xrightarrow{-H_2O} & \\ \text{Pyruvic acid} & \text{Malic acid} \rightarrow \text{Oxaloacetic acid} \rightarrow \text{Pyruvic} + CO_2 & \end{array}$$

The possible pathway of acetoacetate oxidation via conversion to acetic acid has been investigated. Lehninger (107) using muscle minces and cell-free extracts of muscle and kidney reported "a formation of acetic acid from acetoacetic acid which is small and irregular but definite." He proposed three possible mechanisms the evidence being insufficient to choose among them: 1, hydrolysis; 2, phosphorolysis into acetic acid via acetylophosphoric acid; and 3, dismutation of pyruvic acid which may be formed from acetoacetic. Host-Jorgensen (82) discussed the possible formation of acetic acid by dismutation of pyruvic acid which may be formed from acetoacetic acid. Barnes and Lerner (9) studied the metabolism of glycolic and glyoxylic acids in the rat. They concluded that the former is neither glycogenic nor ketogenic. Because the latter causes an increase of blood ketone in the fed rat, the authors suggested that it may be the two carbon unit formed by beta oxidation of fatty acids. Kravel and Gibson (96) were unable to demonstrate oxidation of acetone by liver or muscle in vitro.

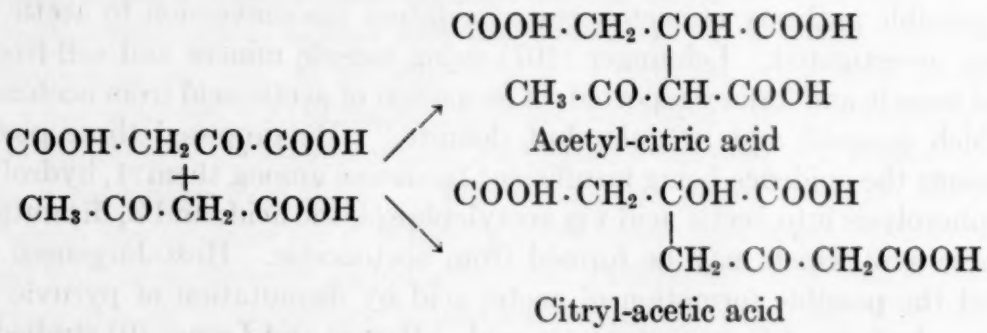
Two highly significant pieces of work have recently been reported which in the main concur. Breusch (22) in a short report without details discussed a new enzyme—citrogenase—which catalyzes the following reaction between beta keto derivatives of fatty acids and oxaloacetic acid:



Beta keto acids (including dicarboxylic acids) are attacked with the formation of citric acid. The degraded residual acid is again oxidized, thus providing for complete oxidation of fatty acids by successive beta oxidations, the intermediates passing through a carbohydrate pathway. The enzyme was demonstrated in large amounts in muscle, kidney and brain, but little in liver, and none in spleen, pancreas or lung. Breusch proposed that oxaloacetic acid is the point common to fat and carbohydrate oxidation as follows:



Wieland and Rosenthal (191), following up the implications of the work of Sunderhof and Thomas (163) with yeast, reported experiments indicating the condensation of acetoacetate and oxaloacetic acid leading to the formation of a procitric acid according to the alternative scheme:



In the presence of kidney brei + barium ions, 80 per cent of the added acetoacetate was converted to citric acid. This they isolated in crystal form and identified. The reaction was demonstrated in heart muscle and kidney. Striated muscle was not investigated, but the liver appeared to be unable to bring about the reaction. However, the authors do not exclude the presence of the enzyme from this organ because its high metabolic activity might possibly have oxidized the citric acid away. The precise rôle of barium or magnesium is not clarified. The two hypothetical pro-citric acids should yield upon hydrolysis acetic acid and citric acid, but the authors were unable to demonstrate the presence of the former. To explain this they assume that the acetic acid condenses with another molecule of oxaloacetic to form citric acid. A peculiarity of the condensation reaction, which should be independent of the oxygen tension, is that in air or nitrogen the synthesis of citric acid is very much diminished (1/10) compared to that in oxygen, and the authors supposed that the synthesis must be coupled with some oxidative process. Analyses other than for citric acid were not reported.

On the basis of these experiments, the authors speculated as to the possibility

that all carbohydrate oxidation goes by this mechanism through acetoacetic acid by way of the established pathways: 2 pyruvic = acetoacetic + carbon dioxide. They even suppose that the effective site of insulin action may be upon this enzyme system. In diabetes the mechanism is impaired and acetoacetic accumulates. But this latter hypothesis is hard to defend: viz. (a) the liver which is the sole site of ketone formation cannot be demonstrated to contain the enzyme; (b) the muscles—which contain the enzyme—do not produce ketone bodies.

Two equally important papers have appeared opposing the conclusions of Breusch and Wieland. Weil-Malherbe (186) cited the following experimental findings: 1, acetoacetate incubated with slices of rat brain or kidney with and without the addition of various members of the citric acid cycle showed insignificant effects on oxygen uptake, formation of citric acid, and disappearance of total ketones bodies; 2, rat brain, testes and kidney equilibrated *anaerobically* with acetoacetate with the addition of oxaloacetic acid showed no significant increase in the disappearance of acetoacetate. The first finding is the precise opposite of the results of Wieland, and the second is covered by the observation of Wieland that the condensation, which should proceed anaerobically, apparently requires oxygen for reasons not apparent at the moment.

The theories of Breusch and Wieland have been even more severely criticized by Krebs and Eggleston (98) in a recent preliminary paper foreshadowing much experimental evidence. The interaction of acetoacetate and oxaloacetate in sheep heart and kidney experiments was studied by a determination of all of the possible reaction products, viz.: acetoacetate, oxaloacetate, β-hydroxybutyrate, citrate, isocitrate, cis-aconitate, α-ketoglutarate, succinate, fumarate, malate, pyruvate, lactate. The authors stated that they have shown unequivocally that the extra acetoacetate removed was fully accounted for as β-hydroxybutyrate. They invoked known reactions which they have demonstrated hitherto, viz., the dismutation of malic or α-ketoglutaric with acetoacetic to form oxaloacetic or succinic together with β-hydroxybutyric acids. Further, oxaloacetic may yield either tricarboxylic (citric, isocitric, cis-aconitic) acids, α-ketoglutaric and malic acids. On this basis they found that their experimental results were readily accounted for by an over-all reaction as follows:

$$2 \text{ oxaloacetic} + 3 \text{ acetoacetic} + \text{water} = \text{succinic} + 3\ \beta\text{-hydroxybutyric} + 4\ CO_2$$

They excluded other reactions and stated that "under the condition of our experiments oxaloacetic acid has no effect on the oxidative breakdown of acetoacetic acid. The intermediate stages of this process must still be regarded as obscure."

The ingredients of a sharp controversy are manifest in these four papers and further reports on this new interesting phase of fat metabolism should be of great interest.

These new facts are of far-reaching importance and point to a possibility of integration of carbohydrate and fat metabolism in terms of fine chemical detail. However, until confirmation and extension are forthcoming, their full implication must remain a matter of very interesting speculation.

ACETIC ACIDS. Although acetic acid is readily oxidized in animals, the exact pathway of intermediary metabolism is unknown. Condensation to acetoacetic acid has already been discussed. The possibility that it may be phosphorylated to acetylphosphate before it undergoes oxidation has likewise been considered (Lipman and Perlman, 112). This raises the interesting possibility that it may be formed in the phosphorylated state by beta oxidation of fatty acids. Hence, it cannot readily leave the cell and engages so rapidly in further catabolic reactions that it never accumulates or spills over into the blood, thus explaining the failure to find it in tissues which are actively oxidizing fatty acids. Kleinzeller (90) from studies with tissue slices, concluded that neither glycolic nor glyoxylic acids could be intermediaries in acetic acid oxidation.

The oxidative condensation of acetic acid through the methyl groups into succinic acid has long been proposed (Thunberg, 178), but there is no proof that this reaction occurs in mammalian tissue. However, its occurrence in bacteria has recently been established beyond reasonable doubt (Slade and Werkman, 160).

Toenniessen and Brinkman (179), in perfusion experiments with muscles and liver, reported that acetic acid is oxidized by the former and converted to ketone bodies by the latter. Formic acid, which is further oxidized, may be a possible degradation product. They deny the possibility of the formation of succinate from acetic acid in mammalian tissue. The oxidation of acetate, glycolate, and glyoxylate has been studied in guinea-pig kidneys by Kleinzeller (90). Acetic acid is more rapidly oxidized by guinea-pig kidney cortex than by any other tissue examined. The oxidation was inhibited by malonate. Glycolic or glyoxylic acids are not significantly oxidized and hence are excluded as possible intermediates.

A most interesting experiment is that reported by Rittenberg and Bloch (143), using acetic acid with C_{13} in the carboxyl and deuterium in the methyl group. They fed rats for a period of 8 days with this preparation, and then studied the distribution of the isotopes in the fatty acids of liver and carcass. They concluded from their data that fatty acids are synthesized by successive condensation of C_2 units.

Wieland, Jennin and Schwartz (190) were unable to show any effect of pigeon breast and leg muscle on acetic acid; it is, however, utilized by the kidneys. The aerobic degradation is retarded by malonate. For this reason the authors concluded that the metabolism goes through the succinate acid cycle. Acetic acid was also utilized by liver and lung tissue. The formation of citric acid or α-ketoglutaric acids could not be demonstrated. Elliot and his co-workers (60) using tissue slices reported acetic acid as utilized by kidney, liver, testis, but not by brain, retina or chick embryo.

Koehler, Hill and Buttenwieser (93) studied the oxidation of acetate injected into normal diabetic subjects. It was rapidly oxidized in both instances, but ketone formation as indicated by urinary estimation was relatively small, being somewhat higher in the diabetics.

Succinic acid. The eagerness of clinicians to seize upon the four carbon dicarboxylic acid as possible therapeutic agents is illustrated by the paper of Koranyi and Szent-Gyorgyi (94). On the basis of the supposed action of succinate in oxidizing away the ketone bodies, they reported beneficial results in the treatment of diabetic ketosis by succinate. The matter was re-investigated by others (51, 117, 177), and the consensus of opinion was that the succinate accomplished no more than an equivalent amount of alkali or glucose.

Iron. A possible relation of iron to fat metabolism is indicated by the paper of Elliott and Libet (61) who studied the respiration of tissue suspensions of brain or liver in the presence of small amounts of iron or an iron-protein complex obtained from the liver. They found, particularly with added ascorbic acid, a marked influence of iron upon oxygen uptake in the presence of mixed phospholipids (cephalin). The significance of this action of iron upon fat metabolism cannot be evaluated without further experiments.

SUMMARY

The intermediary metabolism of fatty acids in mammalian tissue is reviewed under the following heads: 1. Enzyme concerned with fat metabolism. 2. Ketone body production: site of formation; precursors. 3. Chemical mechanisms of ketone production from fatty acids. 4. Ketone formation from acetic acid. 5. Lower fatty acids. 6. Proportion of acetoacetate to β-hydroxybutyrate. 7. Lag in ketone production by liver. 8. Miscellaneous factors. 9. Omega oxidation. 10. Utilization of ketone bodies. 11. Mode of oxidation of fat in muscles. 12. Integration of carbohydrate and fat metabolism. 13. Fat to carbohydrate. 14. Intermediary pathway of fatty acid derivatives. 15. Succinic acid. 16. Iron.

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THE EFFECTS OF DIETARY DEFICIENCIES UPON THE ORAL STRUCTURES

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The oral tissues are peculiarly sensitive to nutritional deficiencies and dietary aberrations. They are often the first to show the effects of such deficiencies, particularly if the latter are subclinical in nature. The oral cavity mirrors the nutritional status of the body because of two unique characteristics:

1. The oral tissues range from the simplest (mucous membrane) to the most highly specialized tissue (the papillae of the tongue and the enamel and dentin). Hard and soft tissues of both epithelial (enamel) and connective-tissue (dentin and bone) origin may be observed side by side. A highly varied range of responses and types of tissue reactions may thus be seen at any one time. In addition, the oral tissues are constantly subjected to trauma and irritation by mechanical, thermal and bacterial agents and therefore are among the first to exhibit the effects of systemic disturbances.

2. The oral cavity is an internal cavity of the body, dark and moist, and lined by a true mucous membrane which is continuous with the mucosa of the gastrointestinal tract. It receives the secretions of specialized glands and plays an important rôle in digestion. Yet of all the internal cavities of the body, it is the only one that is readily accessible and easily examined without the aid of highly specialized instruments.

The oral tissues have been called the barometer of the state of nutrition of the body. Subclinical states are certainly not rare and from the standpoint of diagnosis, perhaps the most important findings are in the mouth (242). The enamel and dentin are kymographic, fixed records of the past history of the individual. The alveolar bone, the gingivae and the tongue reflect the present internal status of the body accurately and quickly. The routine examination of the teeth, the gums, the tongue, the lips and the saliva at periodic intervals is a simple and fairly accurate method of assessing the nutritional status of both the child and the adult.

The reader is referred to Armstrong (5), Marshall (173), Mellanby (186), and Schour and Massler (229) for earlier reviews on this subject.

VITAMIN A DEFICIENCY. The classic work of Wolbach and Howe (276) (278) on the dental changes in vitamin A deficiency has been confirmed and extended in the rat (228) (151) (152) (184) (185) (199), in the guinea pig (200) and the dog (150) (189). Since most of our knowledge of the dental effects of vitamin A deficiency is based on findings in the continuously developing incisors of the rat, the effects in this tooth will be described unless otherwise noted.

It has been well established that the function of vitamin A is concerned primarily with epithelial cells and, in these cells, with the process of differentiation (280). Vitamin A deficiency results in a failure of the epithelial cells to differ-

entiate. The basal cell layer, therefore, loses its type specificity and tends to produce a stratified keratinized epithelium regardless of the type previously formed. In addition, the failure to differentiate allows the basal cell layer to continue its proliferation unchecked. Thus a keratinizing metaplasia results in numerous epithelial structures throughout the body (mucous membranes of the trachea, bronchi, conjunctiva, cornea, accessory sinuses, ureter, vagina, uterus, and periurethral glands as well as the skin). In the developing tooth germ, the first effect of vitamin A deficiency is found in the highly specialized odontogenic epithelium responsible for enamel formation and guiding the formation of dentin.

Odontogenic epithelium. The primary effect is seen as a failure of the young cells of the odontogenic epithelium to undergo normal histo-differentiation and morpho-differentiation (228). This results in a persistence and an increased rate of cell proliferation which in turn leads to an invasion of proliferating epithelial cells into the pulp. This epithelial invasion is very characteristic of vitamin A deficiency (228).

In young rats whose mothers were on a vitamin A deficient diet for five months preceding their birth, the changes are more severe and include a distortion in the shapes of both the incisors and molars (184) (185).

Growing enamel. The mature enamel-forming cells of the odontogenic epithelium (ameloblasts) become small in size and disturbed in function so that the enamel matrix is arrested in its formation and calcification. Enamel hypoplasia is therefore a common finding in the rat following prolonged vitamin A deficiency.

The normal orange pigment of the enamel, which is the final product of the ameloblasts, is lacking and the tooth has a paper-white, unglazed appearance (120) (236).

Growing dentin. One of the functions of the odontogenic epithelium is to organize and cause the differentiation of the subjacent mesenchymal cells of the pulp into odontoblasts. In spite of the fact that vitamin A deficiency affects epithelial cells primarily, the first recognizable histologic change is seen in the mesenchymal odontoblasts. Morphologic changes in the ameloblasts occur much later although their function is affected before the odontoblastic changes are observed.

The odontoblasts on the lingual (cementum-covered) portion of the incisor show an early atrophy and depolarization (278). As a result, the growth of the cementum-covered dentin is seriously affected. It is atypical in structure, lacks the normal tubular arrangement and contains cellular and vascular inclusions. Pohto (199) has emphasized the peg-like dentin projections into the pulp and the prominent foldings.

On the labial (enamel-covered) side of the incisor, the odontoblasts normally differentiate under the influence of the ameloblasts rather than under the less differentiated odontogenic epithelium. The odontoblasts on this side of the tooth appear to be normal and continue to lay down normal dentin long after the odontoblasts on the lingual have disappeared. In fact, the labial dentin becomes excessively wide in contrast to the abnormally thin lingual portion.

The reason for this difference may be a selective alteration in the rate of dentin apposition. Instead of the normal rate of 16 microns per day, vital injections with Alizarine Red S show that the enamel-covered dentin shows an accelerated rate (up to 19 microns per day) and the cementum-covered dentin shows a corresponding *decelerated* rate (down to 6 microns per day) (228).

Pulp. The invasion of the pulp by odontogenic epithelium is very characteristic of a chronic vitamin A deficiency. Amorphous dentin may be formed in the pulp as a result of the organizing influence of this aberrant epithelium upon the invaded pulpal mesenchyme.

Calcification of enamel and dentin. The enamel of the incisors appears dull white and opaque, while the incisal edge is short and blunt, indicating a deficient calcification of the enamel (236).

The dentin shows an interglobular texture. King (151) pointed out that the calcification of the dentin was disturbed in his animals in spite of the fact that they were given ample quantities of vitamin D, calcium and phosphorus. The super-position of a vitamin D deficiency upon a basic diet deficient in vitamin A increases the disturbance in dentin calcification (125) (187) (228) (278).

Vital staining with Alizarine Red S produces a faint red color instead of the normally deep red (228). The teeth contain a lower total ash and a slightly lower percentage of phosphorus but a higher percentage of calcium in the ash (236). All these facts point to a deficient calcification of both the enamel and dentin in vitamin A deficiency.

Alveolar Bone. Vital injections of Alizarine Red S indicate that the alveolar bone is retarded in its rate of formation (228). In the dog, there is an increased density of the alveolar bone with roentgenographic evidence of an ill-defined lamina dura (150).

The cementum is thickened. The periodontal membrane is irregular in width and abnormally enlarged in the fundic region of the rat incisor (228).

Eruption. Eruption is retarded (236) and ceases entirely in prolonged deficiencies (228). In the dog, the teeth are also malposed and show malformed roots (150).

Gingivae. In both the dog and the rat the gingival epithelium becomes hyperplastic and shows a marked keratinization (152) (185) (188) (187). The tissue is easily invaded by bacteria, leading eventually to periodontal disease. In prolonged deficiencies, deposits of calculus occur on the necks of the teeth (152).

Salivary glands. The major and minor salivary glands undergo the typical keratinizing metaplasia that characterizes all the epithelial cells in vitamin A deficiency (280). So far as we could ascertain, an analysis of changes in the quality of the saliva has not been made, although such studies would help clarify the relation of vitamin A deficiency to dental disease.

Other oral changes. Green and Mellanby (102) observed abscesses in the floor of the mouth and suppurating glands in the neck in 72 to 90 per cent of 92 rats deficient in vitamin A.

Effects of Replacement Therapy. Cellular. Wolbach and Howe (278) found regenerative effects within seven days after feeding butter-fat. The odontogenic

epithelium and the odontoblasts resume their normal form and function. The ectopic epithelial cells in the pulp do not differentiate sufficiently to become ameloblasts which might form enamel but regain their organizing influence over the adjacent mesenchymal cells which differentiate promptly into odontoblasts and form dentin (228). Excessive local formations of atypical dentin within the pulp thus increase with recovery. Morphological recovery of the cells is complete within 19 days following replacement therapy (278).

Pigment. Irving and Richards (120) noted changes in enamel pigmentation which varied with different doses of vitamin A replacement.

Rate of growth. Measurements of the rate of dentin apposition by means of vital staining with Alizarine Red S show that the deviation from the normal rate of growth is in direct proportion to the severity and duration of the vitamin A deficiency. The rate of apposition also varies with the amount of replacement with vitamin A. The use of this sensitive dentin reaction as a biologic method of measuring the vitamin A content of foods has therefore been suggested (224)

Tumor formations. Long-continued, chronic vitamin A deficiency is made possible by intermittent feeding of minimal quantities of vitamin A. Orten and Smith (43) observed tumor-like formations (odontomas) in and about the incisors in one-third of their experimental animals. These tumors arise as a result of the scattering of portions of the odontogenic epithelium into the surrounding soft tissues of the jaws. During the feeding intervals these scattered epithelial transplants organize the adjacent mesenchymal cells to form dentin and therefore also odontomas.

Vitamin A Deficiency in Man. Enamel hypoplasia. Vitamin A deficiency may cause a disturbance in the developing tooth germ only in children under six years of age, since after that time the crowns of all the teeth (with the exception of the third molars) are completed. Boyle (31) described changes in the enamel organ of a tooth germ of a human infant suffering from vitamin A deficiency. The changes were similar to those found in the rodent incisor. However, the suggestion that vitamin A deficiency is a common cause of enamel hypoplasia is not supported by findings other than the single case reported by Boyle (31). Bloch (26) (27) studied a number of children suffering from severe xerophthalmia and blindness caused by vitamin A deficiency. He found no dental changes. Investigation on the cause of enamel hypoplasias also does not support the assumption that vitamin A deficiency is a common cause. In fact, in sixty cases of enamel hypoplasia investigated by Sarnat and Schour (216) not a single case of enamel hypoplasia was found to be associated with a vitamin A deficiency.

Oral epithelium. In the adult, the possible effect of vitamin A deficiency is limited to the oral epithelium. Vitamin A deficiency may result in hyperkeratotic changes in the oral mucosa similar to those found on other mucous surfaces. Abels et al. (1) suggest a possible relationship between vitamin A deficiency and hyperkeratosis of the gingivae and oral leukoplakia. Hyperkeratosis of the gingivae has been interpreted clinically as resulting from vitamin A deficiency.

Caries. There is no definite evidence to show that caries is increased because

of a deficiency in vitamin A (60). Administration of supplements of vitamins A and D to children for a period of over one year had no significant effect on caries progress (57). However, the diminished production of lysozyme in most of the body fluids, with resulting loss of bacteriostatic action, may also occur in the saliva (70). The possible rôle of this factor in the saliva as a contributory cause in caries deserves investigation.

Hypervitaminosis A. Significant dental changes could not be found in experimental hypervitaminosis A (280).

VITAMIN B DEFICIENCIES. Unlike the oral findings in vitamin A deficiency which are derived largely from animal experimentation, those in vitamin B deficiencies are for the most part based on clinical observations in man. For this reason, the mechanism of action and many details of the histologic changes in vitamin B deficiencies still remain to be elucidated.

The effects of deficiencies of vitamins A, C and D, and of the minerals, are concerned chiefly with tooth and with bone development. However, deficiencies in the B vitamins are concerned primarily with the oral soft tissues (gingivae, tongue and mucous membranes).

Thiamine (Vitamin B_1) Deficiency. Thiamine deficiency in man is characterized by a marked sensitivity of the oral tissues (171). Weisberger (268) observed small, pinpoint vesicles resembling herpetic lesions on the buccal mucosa, under the tongue or on the palate in elderly patients who were deficient in thiamine. These lesions healed after thiamine therapy. No typical glossitis was observed in experimental subjects studied by Williams et al. (275).

Govier and Greig (100) observed necrotic erosions in thiamine-deficient dogs. However, these lesions healed only after vitamin C was added to the vitamin B replacement. There is therefore reason to question whether these lesions were caused by the thiamine deficiency. The experimental and clinical effects of thiamine deficiency upon the oral tissues need further elucidation.

Riboflavin (Vitamin B_2) Deficiency. Ariboflavinosis is characterized by (1) a magenta-colored glossitis, (2) cheilosis, (3) capillary dilatation and proliferation on the cornea of the eyes, and (4) a seborrheic dermatitis of the naso-labial folds, alae nasi, eyelids and ears (246) (247). When the quartet of these symptoms occurs, the clinical diagnosis of ariboflavinosis is not difficult.

Ariboflavinosis shows early in its course a characteristic magenta-colored, pebbly glossitis which is distinguishable from the scarlet-red, smooth, atrophic glossitis of pellagra and which disappears only after riboflavin therapy (162) (129) (130) (268) (246). Ariboflavinosis also presents a characteristic cheilosis. Sebrell and Butler (232) conducted an experimental study on 18 healthy women placed on a riboflavin-deficient diet. After 94 to 130 days, thirteen developed a fissuring and maceration of the angles of the mouth (cheilosis) resembling perlèche. The lesions responded to additions of riboflavin to the diet but not of nicotinic acid. These findings have been confirmed repeatedly (214) (131) (133). Jones et al. (133) made a careful slit-lamp examination and analysis of the characteristic stomatitis caused by riboflavin deficiency in 1746 men in an African army camp. The stomatitis consisted essentially of a painful glossitis

(not magenta colored) and a painful cheilosis with excessive salivation. The lesions responded to additions of riboflavin or fresh yeast to the diet but not to additions of nicotinic acid.

While the exact mechanism involved in the production of both the glossitis and the cheilosis is somewhat obscure, the clinical stages have been clearly described and are readily recognized.

Glossitis. The glossitis begins in mild deficiencies with a soreness at the tips and/or lateral margins of the tongue. These sites become reddened and glazed as a result of the atrophy of the filiform papillae. The fungiform papillae are engorged and flattened (mushroom shaped), giving the tongue a coarsely granular or pebbly texture. The lesions extend backward over the dorsum of the tongue in the form of ovoid patchy areas of desquamation. In moderate, chronic deficiencies, the entire tongue presents the characteristic granular glossitis. In very severe cases, all traces of the lingual papillae disappear, leaving the tongue smooth, shiny and glazed. Fissures frequently develop. The tongue becomes atrophic with sharp margins indented by the teeth.

Cheilosis. The cheilosis begins as a tiny, raw, red, painful area at the commissure of the lips, at the muco-cutaneous junction. The area becomes larger and is soon covered with a white, adherent epithelial membrane. In severe cases, multiple fissures appear which are very painful. The lesion tends to spread to the lower lip causing a fissuring and cheilitis. It may also spread over the skin but characteristically spares the upper lip. The gingivae are not affected.

Recent findings indicate that the cheilosis may not necessarily be specific for ariboflavinosis but may depend somewhat upon concurrent deficiencies in pyridoxine (238) (169), nicotinic acid (115) or the entire B-complex (129) (130).

Pseudo-ariboflavinosis. Clinically, the glossitis and cheilosis of ariboflavinosis must be distinguished from the atrophic glossitis and pseudo-cheilosis resulting from a closed bite in edentulous persons or in those with poorly fitting artificial dentures (71) (268) (196). Neither should traumatic fissures or monilial infections at the angles of the mouth (perlèche) be mistaken for the cheilosis of ariboflavinosis (244) (130). Sutton and Sutton (245) suggest a causal relationship between cheilosis and perlèche. Although perlèche is a specific monilial infection, it may superpose or follow an underlying ariboflavinosis just as a Vincent's infection may follow and superpose a pellagrous stomatitis.

Effect on bone formation. A possible rôle of riboflavin in skeletal development has been pointed out by Warkany and Schraffenberger (257) who found many congenital malformations, including cleft palate and impaired development of the mandible, in new-born rats whose mothers were subjected to riboflavin deficiency. In a recent abstract, Ross (215) reports a severe type of alveolar-crest atrophy and loosening of the incisors in young adult males suffering from riboflavin deficiency. The rôle of riboflavin in bone growth, however, awaits further investigation.

Vitamin B_2 deficiency in monkeys. Experimental multiple vitamin deficiencies in monkeys produced lesions of the gingivae, the periodontal tissues and the oral mucosa. The most severe changes (including noma) occurred in deficiencies of vitamin B_2 complex (251) (249) (47).

Niacin Deficiency. Glossitis. Niacin deficiency is characterized by a typical, painful, fiery-red glossitis which shows a spectacular and prompt disappearance after administration of nicotinic acid or its amide (144). In the prodromal stage of niacin deficiency, the patients complain of weakness, lassitude, insomnia, headache, anorexia and loss of weight (171). They also complain of a burning sensation in the tongue (glossodynia) and oral mucosa. The tongue is swollen and reddened at the tip and lateral margins and indented by the teeth. The lingual papillae are red and hypertrophied at this stage, giving the tongue a reddened appearance. Kirkland (154) points out that few pellagrins fail to give a history of prodromal painful glossitis, gingivitis and stomatitis. Kruse (163) has described minutely the lingual manifestations as observed with the aid of the biomicroscope in very mild cases of aniacinosis. None of his cases showed skin lesions.

In the acute stages of niacin deficiency, the entire oral mucosa becomes very vascular and fiery red. The tongue becomes tender and painful and the entire mouth feels painfully scalded. There is excessive salivation. In the more advanced cases, the lingual papillae completely disappear, leaving the tongue smooth, dry and glazed. The tongue literally sheds its epithelium, beginning at the tip and lateral margins.

Gingivitis. In contrast to riboflavin deficiency, the gums and gingivae are also attacked. One of the earliest prodromal symptoms of a niacin deficiency is a painful gingivitis and stomatitis. Beginning with the interdental papillae and progressing very rapidly and extensively, the gingivae become very tender and painful and often become ulcerated. The ulcerations usually present a thick, grayish exudate or membrane which gives rich cultures of Vincent's organisms, and may spread to the entire oral mucosa. The Vincent's infection appears in direct proportion to the severity of the deficiency and subsides after adequate niacin therapy (153). King (153) found that the most effective treatment is a combination of niacin and local hygienic therapy.

Dermatitis. In persons with these oral symptoms, the presence of a characteristic dermatitis (confluent red-brown areas with sharp margins, bilateral symmetry and keratosis) is ample evidence of a niacin deficiency (154).

King (153) and Kaufman (144) have shown that an atypical pellagra (pellagra sine pellagra) may occur. The symptoms in these cases are neither as severe nor as constant as in the typical pellagrin and consist principally of vague neuropsychiatric complaints and a relatively mild stomatitis with an atrophic burning glossitis (glossodynia) (172) (50). The symptoms disappear after niacin therapy.

Secondary pellagra. Although the typical pellagrin is not common in the northern states (74), secondary pellagra is rather common and widespread (144) (10). Loss of teeth, partial or complete, dental infections, periodontal disease, tooth extraction, fractures and tumors of the jaws and painful stomatitis are frequently the cause of a secondary pellagra. Such patients are frequently obliged to substitute a soft diet consisting mainly of soups, mush and cooked cereals instead of a balanced one containing meats and uncooked vegetables (10).

Experimental niacin deficiency. Black tongue has been found among dogs

owned by families suffering from pellagra, since these animals live on scraps of the same food their masters eat (241). This condition consists of a glossitis and stomatitis similar to that found in human pellagra. The stomatitis tends to be severe and ulcerative and is quickly cured by niacin (72). The lesions in the mouth often appear before the other general symptoms (66) (70), and appear first as areas of redness on the floor of the mouth, the cheeks or along the inner side of the upper lip. As the deficiency becomes more severe, these areas become dark red and are finally covered with a greenish-gray membrane, due to a superficial necrosis of the oral epithelium. Once the necrosis begins, fetor ex ore and drooling appear. The mouth may become entirely covered with pustules and so foul as to resemble rotten meat (48).

Pyridoxine (Vitamin B_6) Deficiency. Rosenblum and Jolliffe (214) have described a specific edematous magenta-colored glossitis which responds only to pyridoxine therapy. However, the possibility of a concurrent riboflavin deficiency has been considered.

Pantothenic Acid Deficiency. Becks and Morgan (17) and Becks, Wainwright and Morgan (19) describe the effects in dogs of a deficiency of the entire filtrate fraction of the vitamin B complex, which they consider to contain pantothenic acid plus one or more unknown essential elements. The deficiency leads to severe gingival inflammation, necrosis and finally a desquamation of the oral epithelium as well as an osteoporosis and progressive marginal atrophy of the alveolar bone. The supporting spongiosa is porotic and replaced by fat tissue. The clinical and histologic appearance of the alveolar atrophy resembles the atrophic type of periodontosis observed in man. However, the authors caution against drawing generalized conclusions since only one or two animals were used in each experiment and since man may not react as does the dog. Becks and his co-workers feel that all these changes are not caused by a deficiency in pantothenic acid alone since the addition of crystalline pantothenic acid to the diet deficient in the entire filtrate factor did not completely protect the oral tissues against pathologic changes.

Ziskin et al. (286) observed oral lesions consisting of fissures and crusting at the angles of the mouth and ulcers on the buccal mucous membranes and on the tongue in rats on a low pantothenic acid diet supplemented with additions of zinc carbonate.

Vitamin B Deficiency and Oral Disease. Caries. Kniesner, Mann and Spies (159) found a low incidence of dental decay in pellagrins. This is true in spite of the generally poor oral hygiene and high carbohydrate diets. The fact that thiamine is essential to bacterial growth and carbohydrate metabolism may explain the inverse relationship between vitamin B complex deficiency and dental decay.

Periodontal disease. Although the incidence of caries was found to be very low in pellagrins, that of periodontal disease is very high (171) (10).

Oral cancer. Martin and Koop (174) believe that "the most frequent and probably the most important form of chronic irritation, from the standpoint of cancerigenesis in the oral mucous membranes, is that of avitaminosis B." They

also point out that the lesions of avitaminosis B are not confined to the mouth, but affect the entire gastro-intestinal tract and therefore may also play a rôle in the etiology of gastric and intestinal cancer.

Glossodynia. A measurable proportion of patients in the older age groups who complain of abnormal oral sensations (glossodynia and abnormal taste sensations) respond favorably to therapy with the vitamin B complex. Occasional cases respond specifically to niacin or to thiamine therapy. However, in man deficiencies are usually multiple and rarely single.

Vincent's infection. The relation between vitamin B deficiency, more particularly niacin deficiency, and Vincent's infection of the gingivae and other oral tissues, has often been noted (153). Vincent's infection often follows a niacin deficiency but a niacin deficiency is only one of many systemic conditions which predispose to Vincent's infection.

Herpetic infections. Gerstenberger (96) and Burket and Hickman (42) report a prompt and often dramatic response of herpetic lesions to vitamin B complex therapy. In some instances thiamine chloride alone was effective (42). Weisberger (268) observed herpetic-like lesions in thiamine-deficient patients.

Herpetic lesions (herpes labialis, solitary and recurrent aphthae, herpetic stomatitis and herpes genitalis) are specific infections caused by the herpes simplex virus and, like Vincent's infections, are not a specific symptom of vitamin B deficiency. Therefore not all cases will be improved by vitamin therapy (42). The evidence indicates, however, that one of the important predisposing factors in many cases of both Vincent's and herpetic infections is an underlying vitamin B deficiency.

VITAMIN C DEFICIENCY. Vitamin C seems to play a primary rôle in the differentiation of connective-tissue cells, and therefore in the capacity of these cells to form and maintain intercellular substance (8) (277). Those oral tissues which are mesenchymal products (dentin, bone, periodontal membrane) or whose integrity depends particularly upon their vascular supply (gingivae) are primarily affected. The dental changes described below apply largely to the continuously developing incisors of the guinea pig, which has been the animal of choice in studies of scurvy (277) (37) (108). The periodontal changes have been described in man (40) (272) (273), in monkey (250) (251) and in the guinea pig (37).

Pulp and growing dentin. The earliest dental effect is a disturbance in the histo-differentiation of the odontoblasts. These cells atrophy, and become disorientated and are disturbed in function in even mild deficiency states. The vessels of the pulp are dilated and hemorrhagic.

The characteristic disability of the mesenchymal cells to form intercellular substances results in an amorphous and irregular dentin which has been variously called osteodentin, pulp bone and calcific scar (79). The dentinal tubules are few in number and irregularly arranged. Atrophic cells of the pulp may become enclosed in the matrix, simulating the secondary dentin in man. Eventually, the dentin stops forming and as a result the predentin (the newly deposited layer next to the pulp) becomes hypercalcified. This apparent overcalcification of the predentin is secondary to the arrest in dentin formation (280).

Eventually, the odontoblasts become morphologically indistinguishable from other pulpal cells (34). Boyle, Bessey and Howe (37) have pointed out that these changes are not unlike those normally found in the oldest or incisal portions of the continuously growing incisor tooth where the odontoblasts have completed their life-span and undergone senile atrophy. However, in vitamin C deficiency these regressive cellular changes are greater in intensity and occur at the younger, formative end of the incisor.

The histologic changes mentioned above are consistent and so characteristic in their sequence that histologic examination and grading of these changes have provided a biological method for the assay of vitamin C (112) (147).

Fish and Harris (77) believe that the dental changes are the direct result of a primary premature degeneration of the odontoblasts as well as the ameloblasts. They regard the calcified predentin and calcific deposits in the pulp as secondary effects and call them calcific scar tissues. They claim that whenever the odontoblasts degenerate and lose their dentinal fibrils, the pulp attempts to seal off the dead primary dentin by such calcific scar tissue.

Rate of dentin apposition. Boyle, Bessey and Howe (37) found, by means of vital staining with Alizarine Red S, that dentin formation is retarded and eventually ceases in advanced deficiencies.

Enamel epithelium and growing enamel. Atrophic changes in the enamel epithelium and enamel hypoplasia appear considerably later than do the changes in the odontoblasts (33). Boyle (33) points out that the atrophy of the enamel epithelium is caused by traumatic injuries to the enamel organ. The lack of collagen-fiber formation in the periodontal membrane weakens that structure on the lingual surface of the tooth and leads to a failure to resist incisal stresses so that injury to the enamel epithelium on the labial surface results. This is in contrast to the views of Fish and Harris (77) who believe that the atrophy of the enamel epithelium is caused directly by the vitamin C deficiency.

Alveolar bone and periodontal membrane. The osteoblasts lose their ability to form bone and the fibroblasts to form collagenous fibers. This results in a weakening of the structures supporting the tooth and in a porosis and an atrophy of the alveolar bone which is replaced by connective tissue (272) (104).

Occlusal stresses are normally resisted by the fibers of the periodontal membrane. In vitamin C deficiency, the periodontal fibers rupture easily, with the result that the tooth impinges upon the weakened, atrophic alveolar bone and causes its resorption. The teeth thus lose their ability to withstand normal occlusal stresses, become loose and may be easily extracted (35). These changes have been considered to be similar to those characteristic of systemic periodontosis (diffuse alveolar atrophy) in man (35) (40).

Gingivae. In the continuously growing incisors and molars of the guinea pig the periodontal tissues are replaced very rapidly so that the scorbutic effects are usually not as outstanding as they are in monkeys and in man. In addition, the striking changes in the pulp and growing dentin have diverted attention from the changes in the periodontal tissues. Boyle, Bessey and Wolbach (35) observed that the gingivae of the scorbutic guinea pig were congested but that there were no pocket formations.

Westin and Kalnins (274) succeeded in producing scurvy in the rat in spite of the ability of this animal to synthesize vitamin C. They describe severe disturbances in the periodontal tissues of both the continuously growing incisors and the molars which, like those in man, are of limited growth. They observed a severe marginal gingivo-alveolar osteitis and osteomyelitis, the extent and severity of which were in direct proportion to the length of the experimental period. In 162 days of vitamin C deficiency, the alveolar process around the molars was completely destroyed and the teeth were lost. The changes in the incisors, because of their continuous growth and development, were much less severe.

Eruption. Dalldorf and Zall (54) have reported retardation in the eruption of the incisors of guinea pigs. They and others (69) suggest the use of the rate of eruption as an accurate index of the degree of scurvy.

Changes in monkeys. Tomlinson (250) observed a marked hyperemia and hypertrophy of the gingivae in four vitamin-C-deficient and four vitamin-C and calcium-deficient monkeys. The free margin of the gingivae became so hypertrophied as to cover the entire crown of the teeth. Food debris tended to accumulate and produced a foul odor. In severe cases, areas of the free gingivae became necrotic and the teeth became loose. Subperiosteal hemorrhages lifted the soft tissue from the underlying bone.

The hemorrhage and necrosis extended for a short distance into the periodontal membrane which was markedly deficient in fibers. The alveolar bone was thin and showed many subperiosteal hemorrhages and abnormal resorptions but no necrosis. The bone marrow was edematous and contained an increased number of young fibroblasts but was markedly deficient in fibers. Extensive hemorrhages also occurred in the joints, including the temporo-mandibular joint, and in the growing epiphyseal plates, causing the latter to separate and eventually disappear.

Those animals which were placed on a diet deficient in both vitamin C and calcium showed less severe changes than those deficient in vitamin C alone. Vitamin C therapy relieved the earlier symptoms but there was no recovery of the interdental papillae or other tissues which had advanced to the stage of necrosis, nor was there a tendency to reattachment of the separated gingivae (86) (87).

Factors Influencing the Effects of Vitamin C Deficiency. Increased stress. Dalldorf and Zall (54) observed more advanced changes when the teeth of vitamin C deficient animals were under increased occlusal stress. This factor is particularly important in man.

Pregnancy. Scorbutic guinea pigs show more pronounced changes during pregnancy. The effects are more severe in the continuously growing teeth of the mother than in the young (56). The relation of vitamin C deficiency to pregnancy in man has not been adequately studied.

Toxins. King et al. (148) found that the administration of diphtheria toxin to guinea pigs caused marked injury to the odontoblasts and to dentin formation. Administration of 5.0 mgm. of ascorbic acid per day protected against this injury.

The effect of toxic states in man in the production of dental and oral lesions has been noted often but has not been adequately studied, particularly in relation to increased vitamin C needs.

Replacement therapy. Boyle, Wolbach and Bessey (34) observed that the pulpal cells differentiated promptly after even partial replacement therapy. However, instead of the normal dentin deposited in normal incremental layers arranged parallel with the pulpal surface, an irregular, bone-like dentin is deposited in the pulp in the form of long spicules which project toward the center of the pulp cavity. These spicules represent a partial recovery of the dentin-forming cells to form dentin. Where the odontoblasts had survived the vitamin C deficiency and the replacement was adequate, dentin formation became normal in structure and proceeded at a normal rate (34).

Boyle, Bessey and Howe (37) found that in vitamin C deficient guinea-pigs, the amount of dentin deposited in the formative end of the incisor varies directly with the amount of ascorbic acid administered. They therefore suggest the possibility of utilizing this reaction as an objective, biologic assay for the determination of vitamin C content in foods.

Vitamin C Deficiency in Man. Growing teeth. Boyle (32) examined the teeth of two scorbutic infants and found none of the changes described in the growing dental tissues of a scorbutic guinea pig. One infant showed small cysts in the enamel organ and minute hemorrhages. Boyle (32) explains the absence of the characteristic changes on the basis that human teeth grow much slower and that unerupted teeth are free from functional stresses. There may of course be a basic difference in the reaction of human and other species. There has been no human demonstration of the changes observed in the growing dental tissues in the guinea pig (279).

Eruption. Pediatric texts frequently state that the eruption of teeth is delayed in scorbutic infants. In our experience, scorbutic infants show an accelerated eruption of the teeth.

Adult teeth. Westin (272) (273) made a detailed and comprehensive histopathologic study of 18 human adults suffering from various degrees of scurvy. The enamel showed no changes. The pulp was hyperemic and edematous and contained various formations of secondary dentin. Calcified or necrotic areas were also present. The odontoblasts showed degeneration. The dentinal tubules underwent porotic changes which Westin regards as dentinolysis.

Gingivae. The effects of vitamin C deficiency in man are confined largely to the continuously growing periodontal tissues and rarely affect the enamel or dentin. When the teeth are not yet erupted or have been extracted, the marginal gingivae, the alveolar crest and the organized periodontal membrane are nonexistent and therefore the effects on the periodontal tissues are absent. Unlike vitamin B deficiencies, scurvy rarely affects the tongue, lips or buccal mucosa.

Scorbutic gingivitis. The first clinical evidence of latent scurvy in man is seen as a hyperemia and swelling of the interdental and marginal gingivae which become very tender and bleed upon the most trifling trauma (164).

In mild acute cases the subsurface capillaries become engorged and dilated so

that the gingivae become violently reddened and in moderate cases swollen, giving the surface a smooth, shiny, dark-red appearance. The process begins on the interdental papillae and soon involves the marginal (free) gingivae, forming a swollen collar around the teeth which bleeds very easily upon slight trauma and is sharply demarcated from the purplish alveolar (attached) gingivae. The gingival crevice is enlarged to form a pocket which soon is filled with debris and calculus and which in turn irritates the gingivae and aggravates the condition.

In fully developed acute scurvy, the gingivae become boggy and are soon denuded of epithelium, showing dilated, thin-walled, easily ruptured blood vessels. The color of the gingivae changes rapidly to a violaceous red. The alveolar (attached) gingivae may also become involved until the gums are very much swollen. In infants, the swollen tissue may entirely cover the newly erupted deciduous teeth. In severe acute cases the hemorrhage tends to be spontaneous and the pain is constant and intense. The gingivae may become ulcerated and secondarily infected with Vincent's organisms.

In mild chronic scurvy, the subsurface capillaries undergo relatively slight dilatation and engorgement. The interdental papillae and the marginal gingivae become swollen and edematous but not reddened. Atonia of the marginal gingivae is very marked so that they tend to fall away from the teeth. This results in gingival pockets and the accumulation of débris from which a distinctly foul odor emanates. The marginal gingivae form a spongy and edematous collar which tends to recede from the necks of the teeth.

In severe chronic cases, the swollen gingival collar may become covered with small ulcers and granulation tissue. At the same time, hemorrhages and swelling of the periodontal membrane occur and are followed by rarefaction and loss of the alveolar bone. As a result, the teeth soon become loosened and fall out.

Kruse (164) gives an excellent and detailed description of the above changes in acute and chronic scorbutic gingivitis as seen with the aid of the biomicroscope.

Local factors, particularly trauma, calculus and malhygiene, undoubtedly play a significant rôle in the progress of the scorbutic gingivitis and the periodontal breakdown, since these lesions begin and are obvious only at sites of irritation and stress. They do not occur at all if the teeth are unerupted or absent. A similar situation obtains in other types of systemic gingivitis and periodontal disease, such as lead lines, diabetic gingivitis, etc. (231).

Vitamin C Deficiency and Dental Disease. Gingivitis. Gingivitis is a very common disease which affects more than 75 per cent of all adults to some degree. It is caused for the most part by local factors such as malhygiene, calculus and trauma. However, in view of the facts that vitamin C deficiency predisposes to gingival disease and that latent scurvy shows a high prevalence (30–65 per cent) among persons who are on an apparently adequate diet (283), the possible systemic rôle of vitamin C deficiency in gingivitis cannot be neglected.

While vitamin C deficiency has frequently been cited as an underlying cause in gingivitis (164) (44) (28) (243), there are many investigators who found no correlation between low blood plasma levels of ascorbic acid and the average case of gingivitis (202) (204) (203) (107) and many who obtained no therapeutic

results from large oral doses of vitamin C (59). Moreover, in spite of the recognized increase in vitamin C intake in the United States, there has been no apparent decrease in the incidence of gingivitis (201). On the other hand, when the gingivitis is caused by a latent or subclinical but demonstrable vitamin C deficiency, excellent results follow treatment with ascorbic acid (205), (243) (145).

Additional and better controlled investigations are needed to supply conclusive evidence on this important but controversial problem.

Vincent's infection and scorbutic gingivitis. A secondary Vincent's infection maybe superposed upon an underlying scorbutic gingivitis (243) (145). The scorbutic gingivitis is distinguished from a primary Vincent's infection by the fact that (a) the ulcers are small and localized to the marginal gingivae, (b) there is no lymphadenopathy, and (c) there is a dramatic response to vitamin C therapy (145).

Periodontal disease (alveolar bone and periodontal membrane). The possibility of vitamin C deficiency as the etiologic agent in periodontal disease has been pointed out many times (40) (272) (273) (28) (46) (267) (35) (36) (110). However, vitamin C therapy in patients suffering from periodontal disease has not been attended with conspicuous success (202) (44).

Boyle, Bessey and Wolbach (35) point out the similarity in the histopathology of the attachment apparatus between both experimental and clinical scurvy and diffuse alveolar atrophy. Westin (272) (273) describes the changes in the periodontal tissues in human scurvy as *1*, marked widening of the periodontal space; *2*, resorption of the cementum and alveolar bone; *3*, hemorrhage; *4*, osteoporosis and disappearance of hematopoietic tissue. There is no pocket formation.

Weisberger et al. (267) reported direct correlation between low blood ascorbic-acid levels and periodontal disease. Therefore Boyle, Bessey and Wolbach (35) recommended blood tests for ascorbic acid in all cases of periodontal disease. However, negative findings have also been reported (44), (202).

In a study by Crandon, Lund and Dill (53) one of the authors subjected himself to vitamin C deficiency and found that the earliest changes that could be observed were in the dental roentgenograms which showed slight but definite interruptions in the lamina dura (alveolar bone proper). These authors therefore suggest the possibility that roentgenographic observation of the lamina dura be used as a criterion in the diagnosis of incipient scurvy.

There is no doubt that vitamin C is as necessary for the maintenance of periodontal tissue as it is for rapid wound healing. However, although the relation of vitamin C deficiency to gingivitis may be postulated on the basis of a sub-clinical deficiency, periodontoclasia is a rather severe type of tissue destruction and should be attended by other evidences of a frank deficiency before it may be regarded as a disorder of scorbutic origin (203).

Caries. Claims that caries may be controlled or arrested by vitamin C therapy have not been substantiated. As far as can be determined, there is no relationship between vitamin C deficiency and caries (272), nor is vitamin C therapy

effective in arresting caries (101). Westin (272) found no case in either guinea pigs, monkeys or man, in which scurvy had been a determining factor in the development of caries. Grandison, Stott and Cruickshank (101) found no significant effect on dental caries following a daily administration of 200 mgm. of synthetic vitamin C to 20 children for two years.

Vitamin C in extraction wound healing. Campbell and Cook (46) observed in eight cases that vitamin C given before, during or immediately after tooth extraction dramatically accelerated the healing of the extraction wounds. In addition, they observed less postoperative pain and bleeding.

VITAMIN D DEFICIENCY. Vitamin D is concerned primarily with calcium metabolism and the calcification of the hard tissues. Deficiencies in vitamin D are therefore reflected as deficient calcifications of the hard tissues—growing enamel, dentin and alveolar bone. The changes in the rat following a diet deficient in vitamin D (but high in calcium and low in phosphorus) were recently studied in detail by Weinmann and Schour (262–266).

Growing dentin. The earliest effect of an acute deficiency in vitamin D is the appearance in the dentin of a line of disturbed calcification (calcio-traumatic line) (262).

The response of the growing dentin to even a slight deficiency in vitamin D is so delicate that it can be used as an indicator of the adequacy of the vitamin D in the basal diet (218). Calcification of the dentin occurs by the coalescence of individual small calcospherites which begins at the predentin-dentin border. In even mild deficiencies, the coalescence of the calcospherites is incomplete so that instead of a homogeneously calcified dentin, interglobular dentin results. The dentin remains acidophilic and is dotted with the basophilic calcospherites instead of being homogeneously basophilic. The normally sharp demarcation between the predentin and calcified dentin therefore also disappears.

In more severe chronic deficiencies of vitamin D, the predentin does not become calcified at all, so that as the dentin matrix continues to be deposited, the uncalcified predentin border becomes wider and wider until in severe deficiencies it may attain a width of 90 microns instead of the normal 10 to 20 microns (142) (262). In very severe, prolonged deficiencies, pulpal inclusions may be found in the newly formed dentin (123) (11) (262). The rate of dentin formation is retarded (262).

Pulp. Atrophy of the odontoblasts has been reported by Becks and Ryder (11), but has not been confirmed. Blackberg and Berke (24) (25) observed inflammatory changes in the pulp. Secondary dentin is also retarded in the rate of formation and disturbed in calcification (187) (190).

The effects of a vitamin D deficiency are markedly aggravated by (a) a deficiency in calcium and/or phorphorus (127) (168) (11), (b) a disturbance in Ca/P ration (127) (187) and (c) a deficiency in vitamin A (187) (125). In each instance the primary effect on the hypocalcification of the dentin is exaggerated.

Growing enamel. Enamel formation and calcification are apparently unaffected by vitamin D deficiencies. The enamel organ shows no changes during enamel formation. However, when the enamel organ reaches the stage when it is ready to atrophy, cystic degenerations may occur (262). This effect

takes place only after the enamel is completely formed and calcified (262). Apparently, enamel hypoplasia results only when the vitamin D deficiency is aggravated by a direct or indirect deficiency in calcium (187) or by parathyroidectomy (157). Becks and Ryder (11) did find enamel hypoplasia in rats subjected to vitamin D deficiency but it is likely that this deficiency was aggravated in their animals by some unknown factor (95).

Alveolar bone. The newly deposited layer of bone is unable to calcify in vitamin D deficient animals, and so remains acidophilic and the normally thin osteoid border becomes very wide (11) (263). This uncalcified osteoid tissue also resists resorption (264). Weinmann and Schour (263) explain the increased thickness of the rachitic bone on the basis of a generalized lack of resorption and do not accept the explanation suggested in the term "compensatory hyperplasia."

Periodontal membrane. The periodontal space is narrowed and compressed by the continued growth of the osteoid border of the alveolar bone and its lack of resorption, and eventually undergoes hyaline degeneration. In the bifurcation of the rat molar, the periodontal membrane is crushed out of existence by the osteoid bone so that the latter is often in contact with the cementum (11) (263).

Cementum. The formation of cementum matrix is normal but its calcification is defective so that the uncalcified precementum border becomes very wide (73).

Eruption. Eruption of the teeth is retarded (263) (109).

Changes in animals other than the rat. Changes essentially similar to those observed in rats have also been found in the growing dentin of dogs (187) (191) (24), and guinea pigs (116). In puppies fed a diet deficient in vitamin D and rich in cereals, the secondary dentin normally formed in response to caries or attrition is either absent or reduced in amount and imperfectly calcified (187). Becks and Weber (12) found osteoporosis and loss of supporting alveolar bone in dogs placed on a diet deficient in vitamin D. In dogs placed on a diet low in vitamin D and aggravated by low calcium and high phosphorus, they observed a replacement of bone by fibro-osteoid tissue and an obliteration of the lamina dura and periodontal membrane.

Replacement Therapy in Rickets. Vitamin D. Irving (123) and Weinmann and Schour (265) treated vitamin D deficient rats with vitamin D and observed an improvement in the calcification of the newly formed dentin. The predentin becomes narrowed and sprinkled with calcifying globules and the healing occurs first in the oldest layers of the predentin, next to the calcified dentin (265).

Phosphates. Administration of phosphate to vitamin D deficient rats results in a resumption of calcification in the oldest layers of the osteoid tissue at the end of the second day. Osteoclasts reappear and resorption of the newly calcified tissues occurs (266). The dentin also shows improved calcification after phosphate therapy and a recovery to a normal rate of formation.

Starvation. Starvation of rachitic rats also produces a healing effect in the dentin (265). The possible effects of spontaneous fasting of animals during short-term experiments must therefore be kept in mind in evaluating disturbances in calcification.

Vitamin D Deficiency in Man. Growing dentin. Typical rickets does not

occur except in the young growing organism with a definite need for calcium for the calcification of its growing hard tissues. The histologic changes in human teeth are similar to those seen in experimental animals and consist of deficient calcification of the growing dentin (interglobular dentin and a wide predentin) (98) (281) (139). A disturbance in the calcification of the dentin is not necessarily accompanied by a similar disturbance in the enamel (137). This has already been pointed out in experimental vitamin D deficiencies (262).

There is apparently a selective action of vitamin D in the calcification of the mesenchymal hard tissues (bone and dentin) since deficiencies in vitamin D do not usually affect the enamel.

Enamel hypoplasia. Vitamin D deficiency is generally believed to cause enamel hypoplasia in man. A review of the experimental and clinical evidence does not allow an uncritical acceptance of this view. In sixty cases of enamel hypoplasia, only ten patients had a history of rickets (216). Friel (89) reports an incidence of 28 per cent of enamel hypoplasia among Vienna children who suffered from a rachitogenic and otherwise deficient diet after the First World War. Infantile rickets does not always result in enamel hypoplasia and it is likely that, as is observed in experimental animals, enamel hypoplasia occurs when the vitamin D deficiency is aggravated by some other condition such as infantile tetany on a calcioprivic or parathyroprivic basis (235).

Eruption. The eruption of the deciduous and permanent teeth is retarded (89) (239).

Malocclusion. Malocclusion of rachitic origin has been reported. The imperfectly calcified alveolar bone is unable to withstand normal oral stress and therefore the teeth become maloccluded.

Vitamin D deficiency and caries. A number of clinical investigations have been conducted on the effect of vitamin D on caries, with conflicting results. Mellanby and her co-workers (190) (191) (192) have repeatedly suggested that optimal amounts of vitamin D will reduce the incidence of dental decay, although they were unable to produce dental caries in their experimental animals that were placed on a vitamin D deficient diet. McBeath and Verlin (176) reported a lower incidence of decay in children receiving a daily addition of 800 units of vitamin D in their diet. These findings, however, are not generally supported since many authors found no effect on caries when supplements of either calcium and/or vitamin D are added to the diet (170) (57). In spite of the increased intake of vitamin D in children during the last two decades, the incidence of decay has not shown any decrease (39). In addition, it must be emphasized that rickets is not necessarily accompanied by dental caries (89) (60). Youmans et al. (284) observed no relation between extensive dental caries and calcium or vitamin D intake.

HYPERVITAMINOSIS D. A considerable literature has been developed on the deleterious dental effects of overdosage with vitamin D (105) (260) (18).

Growing dentin. Harris and Innes (105) report irregular calcification of the growing dentin in rats given excessive doses of vitamin D. A detailed histologic study correlated with blood calcium findings of the effects of single massive doses of vitamin D showed, in the dentin, an immediate *primary* reaction in the form of

a poorly calcified layer and a *secondary* reaction in the form of a well or excessively calcified layer (220). Schour and Ham (220) related the *primary* response to the rise in blood calcium and the *secondary* reaction to the *return* of the blood calcium to normal. Similar results were observed following the administration of parathyroid hormone (222). No effect was observed in the growing enamel.

Massive doses of calciferol administered to parathyroidectomized rats resulted in an improved calcification of the dentin similar to the effect produced by the treatment with parathyroid extract (222). While the influence of vitamin D and the parathryroid gland upon the calcification of the teeth can hardly be questioned, the mechanism of their action and their possible interrelationship present unsolved problems for future research.

Periodontal structures. The alveolar bone becomes hypercalcified after massive doses of vitamin D. The cementum is hyperplastic as well as hypercalcified so that in the molar teeth a partial obliteration of the periodontal membrane results (105) (260) (77).

In dogs, feeding of excessive amounts of vitamin D (with optimum amounts of vitamin A) results, according to Becks (18) in: (1) generalized osteosclerosis of the jaws and alveolar bone; (2) severe pathologic calcification in the periodontal membrane and gingivae; (3) excessive formation of an atypical cementum leading to ankylosis between tooth and bone; (4) multiple pulp stones, and (5) extensive formation of calcium. The addition of excessive amounts of vitamin A *lessened* the injurious effects.

The rate of dentin and bone apposition, as measured by vital staining with Alizarine Red S was significantly increased following the daily administration of 78,000 U.S.P. units of vitamin D per kilogram to rats for a period of two weeks (285).

VITAMIN E DEFICIENCY. Prolonged deficiency of vitamin E in the rat results in a loss of pigmentation of the enamel of the incisor (55). Davies and Moore (55) explain this on the basis of a secondary deficiency of vitamin A, although whitening of the teeth has been observed in rats suffering from vitamin E deficiency but who still had considerable vitamin A in the reserve. Irving (124) found in addition a premature and abnormal degeneration of the enamel organ which he regarded as being possibly specific for vitamin E deficiency. It must be pointed out that premature atrophy of the enamel organ is common in many chronic disturbances.

MINERAL DEFICIENCIES. *Total Salt Deficiency.* Rats placed on a diet very low in all inorganic salts show severe disturbances in the calcification of the growing teeth and of the bone (7) (2).

The predentin becomes wider than normal and the amount of dentin formed becomes decreased until the incisors are mere shells and fracture readily. In prolonged deficiencies, dentin formation is irregular and vascular (pulpal) inclusions occur. In the molars, secondary dentin formation becomes irregular and pathologic cuspal fractures occur, followed in turn by carious lesions, pulpal necrosis and periapical abscesses. Arnim, et al. (7) point out the fact that no enamel hypoplasias occurred in their animals.

The alveolar bone is more severely affected than is the dentin. The bony

trabeculae of the supporting spongiosa disappear and are replaced with fatty marrow tissue until only a thin shell of bone is left to support the teeth (7).

Calcium and Phosphorus Deficiency. Rats placed on a diet deficient in calcium and phosphorus show disturbances in the calcification of the growing dentin and bone with but little effect upon the growing enamel (Gaunt and Irving, 1940).

Growing dentin. Calcium and phosphorus deficiency results in a progressive increase in the width of the predentin, an irregular border between the predentin and dentin, interglobular dentin and, in severe chronic deficiencies, vascular (pulpal) inclusions (93) (38) (270). The width of the predentin, normally 10 to 20 microns, may reach 90 to 100 microns in direct ratio to the degree of deficiency and is a much more sensitive measure of the deficiency than the chemical (ash) analysis (92) (93) (143) or the blood calcium analysis (22).

Growing enamel. Gaunt and Irving (95) found no changes in the enamel organ or in enamel formation or calcification. However, Boyle and Wesson (38) employing a low-calcium diet with a P:Ca ratio of 16 to 1 observed premature atrophy of the enamel organ and, in severe chronic cases, disturbed calcification and hypoplasia. Supplements of vitamin D prevented these effects (270) (38). Thus it is clear that in calcium and phosphorus deficiencies, as in vitamin D deficiency, the growing enamel is much more resistant to disturbances in calcification than are the growing dentin and bone.

Alveolar bone. The alveolar bone tends to show excessive resorptions (13) (12). This is in contrast to vitamin D deficiency in which resorptive activity is decreased because of the osteoid border (263). In calcium and phosphorus deficiencies, the effect upon the bone is seen before the effects can be noted in the dentin (7) (93).

Alterations in Ca:P ratio. For the normal calcification of dentin and bone, the Ca:P ratio must be between 4.0 and 5.0 and the amount of these elements in the diet must be at least 0.3 per cent (95). If the calcium and phosphorus content of the diet is less than 0.3 per cent, rickets results. The effect of a deficient intake is aggravated by changing the ratio of Ca:P. Downs (67) observed disturbances in calcification when the Ca:P was either abnormally high or abnormally low. Gaunt and Irving (95) showed that when the Ca:P ratio is lowered from 4.0 to 1.0 or 0.5, the effects upon the dentin are much more severe than upon the bone. Vascular inclusions are common and the ash content of the bone is much less affected. On the other hand, when the Ca:P ratio is high but deficient in amount the effects upon the bone are more severe than upon the growing dentin (95).

Vitamin D therapy in calcium deficiency. Vitamin D therapy greatly lessens the effects of a diet deficient in calcium (38) (270). Boyle and Wesson (38) suggest, but do not prove, that the action of vitamin D in improving the calcification of tissues in animals on a low-calcium diet is due to an increased retention of calcium. They also show that a high protein diet limits the effectiveness of the vitamin D.

In rats on a calcium-phorphorus deficient diet, Irving (127) observed a resumption of calcification in the newly formed dentin 30 hours after the administration

of 18.4 i.u. of vitamin D. The amount of vitamin D necessary for the resumption of good calcification varied with the Ca:P ratio of the diet.

Multiple deficiencies in vitamin D and calcium. Vitamin D deficiency greatly aggravates the effects of a calcium-deficient diet (187) (127). Lund and Armstrong (168) fed rats a diet low in calcium and essentially vitamin D free. They observed, on gross dissection, that the alveolar bone was soft and friable and the molars were loose. The incisors were well developed. Becks and Weber (12) observed marked resorptions and osteoporosis in the jaws of pups fed a diet deficient in vitamin D, low in calcium but high in phosphorus. The supporting bone was entirely replaced by fatty marrow. Toverud and Toverud (252) fed pups a diet deficient in calcium, phosphorus and vitamins A and D and found that the age of the animal and the duration of the experiment influence the severity of the results. The disturbances in the calcification of the teeth were increasingly severe if the experimental diet began at the end of lactation, at birth or during gestation.

Parathyroidectomy also aggravates the effects of a diet low in calcium (22).

Difference in effect of calcium deficiency on dentin and bone. The effect of a calcium-deficient diet is much less severe in the growing dentin than in the bone (93). Dentin has a distinct priority for the available calcium since in mild deficiencies, dentin calcification may be normal while bone calcification is deficient (142). Erdheim (73) points out that certain tissues are less susceptible to disturbances in calcium metabolism than are others. His calcioprotective law has been proved many times (7) (92) (49) (217). In addition, while bone may show marked resorptions and replacement by fatty marrow, the dentin does not. Dentin is not subject to calcium withdrawal by resorption, as is the bone (226). In fact, the calcium and phosphorus made available by the bone are avidly taken up by the calcifying enamel and dentin (227).

Adult teeth. Efforts to alter the calcium content of the adult dentin in dogs by large doses of vitamin D and calcium over periods up to 135 days have been unsuccessful in spite of the fact that the calcium content of the blood was almost double the normal (78).

The molars of rats placed on a diet deficient in calcium show that the preexperimental dentin is unaffected, but the secondary dentin, forming during the experimental period, is poorly formed and calcified (38).

Calcium deficiency in man. Calcium is an important dietary consideration and deficiencies in calcium intake are very common in man (92) (93) (284). The changes in the growing dentin and bone are similar to those observed in experimental animals (175) (81). The fully formed enamel and dentin are not affected by calcium deficiencies or by any calcioprivic state (225).

Roentgenograms of the growing epiphyses readily reveal deficiencies in calcium and vitamin D in the growing child. This method of assessment is not applicable in the adult.

Since, however, the alveolar bone continues to grow throughout life and is composed, like the trabeculae under the epiphyseal plate, of spongy bone, dietary deficiencies related to mineral metabolism affect this bone quickly. On the basis

of work now being conducted, the authors feel that intra-oral dental roentgenograms of the alveolar bone may show the effects of calcium deficiencies in the adult.

Calcium and caries. There is no established relationship between calcium therapy or calcium intake and caries (63) (170). Day (60) found that rachitic children and women suffering from osteomalacia in Northern India were remarkably free from caries. Malan and Ockerse (170) studied a group of children who were fed daily supplements of calcium and phosphorus (0.5 gram each) and found no significant effect on the incidence of caries. Rats on low-calcium diets do not show any increase in caries (38). Day and Sedwick (57) found that supplements of vitamin D had no significant effect on the incidence of caries. Biochemical studies have shown that caries is not dependent upon the calcium content of the enamel or dentin (4).

Iron deficiency. The oral manifestations of iron deficiency have been given much less consideration than those of calcium deficiency and merit investigation.

Magnesium deficiency. Rats placed on a diet deficient in magnesium show characteristic disturbances in both the calcification and the formation of the incisors. Radiographic findings consist of a widening of the periodontal membrane, an indistinct lamina dura and a disturbed contour of the enamel surface (90). Eruption is retarded to one-third the normal rate (91) (68). Chemical studies show no change in the absolute amount of magnesium in the incisors, in contrast to the long bones which show a decrease (68).

Calcification of dentin. Disturbances in the calcification of the dentin are characterized by a prominent stratification which is unlike that seen in any other known experimental condition. The striations are sharply delineated and are repeated at intervals of 32 to 48 microns (121). It has been assumed that these striations are associated with the intermittent interference with calcification and the convulsive attacks of magnesium deficiency (91). However, Watchorn and McCance (258) also found these striations in subacute magnesium deficiency in which no convulsions occurred.

Formation of dentin. The rate of dentin apposition is progressively decelerated, the enamel-covered dentin being retarded almost immediately and more severely than the cementum-covered portion.

The widths of the labial and lingual dentin are approximately the same in the normal animal. In magnesium deficiency, the labial width is one-third or one-half that of the lingual width. The pulpal outline, therefore, is characteristically distorted. This finding is in direct contrast to that in vitamin A deficiency in which the rate of formation of the enamel-covered dentin is accelerated and the cementum-covered dentin is decelerated.

Temporary local cessation of dentin growth may occur. The rate of formation is also decreased in the growing dentin of the molars. The retardation of the rate of dentin apposition and tooth eruption is greater than that of body weight (90) (91).

Pulp. The odontoblasts show an early and progressive atrophy. They are

reduced in size and become enclosed and calcified within the dentin matrix (258). The pulp shows various types of calcification (16).

Growing enamel. The enamel epithelium undergoes early atrophy and degeneration and in advanced stages of the deficiency, the enamel is severely hypoplastic (15). Irving (121) describes calcareous granules which become embedded in the enamel matrix at the basal end.

Periodontal structures. Klein, Orent and McCollum (158) reported a gross proliferation of the periodontal structures in their rats. The gingivae were hypertrophied and the periodontal membrane showed edematous changes in the intercellular structure and an increased width. The growth of the alveolar bone is decelerated to one-half or less of the normal rate (91).

Replacement therapy does not change the structures already formed during the deficiency but leads to a recovery of the odontoblasts and to normal eruption.

Fluorosis. Although fluorosis does not constitute a dietary deficiency, the presence of fluorides in the drinking water (and to a much lesser extent, in foods) of many communities and its effects on tooth development call for its consideration in this review. There is no evidence that fluorine is *essential* to the body economy or to tooth development. In fact, fluorine is an enzymatic inhibitor and a protoplasmic poison. Fluorides exert a harmful effect when ingested in *excess* amounts, in contrast to most nutritional factors which affect the teeth and periodontal structures when they are deficient in amount.

Fluorosis has been the subject of comprehensive reviews (195) (206) (103).

Growing enamel. The developing enamel is the first structure to react to the ingestion or injection of fluorides. The ameloblasts show a selective response in the form of abnormal globules within their cytoplasm as early as one hour following a single injection of 0.3 cc. of a 2.5 per cent solution of NaF (219). This is followed by the formation of sharp rings of disturbed calcification in the calcifying enamel and dentin. The disturbance in calcification is probably brought about by the deposition of calcium fluoride in the tissue instead of the normal calcium salts (PO_4 or CO_3) and possibly also by disturbances of the enzymatic phosphatase system concerned in calcification (65). Irving (126) suggests that fluorine acts primarily on the composition of the blood.

The demarcation of the growing enamel and dentin by injections of sodium fluoride permits an exact quantitative measurement of the rate of growth of enamel and dentin in animals and man (218) (219) (223) (127).

Sodium fluoride fed to rats results in different degrees of disturbance in the calcification of the enamel, depending upon the dosage and duration of the experiment. The effects range from mild disturbances such as the loss of pigmentation and defective calcification in the enamel to severe enamel hypoplasias (230).

In man, the ingestion of water containing more than 5 parts per million of fluorides during the period of enamel (crown) formation and calcification results in a characteristic mottled enamel in 90 per cent of children. In 35 per cent the enamel may be subject to hypoplasia and is permeable to oral stains so that it

often appears brown. The ingestion of water containing about 2 p.p.m. of fluorides results in mild mottling in approximately 50 per cent of the children. The ingestion of water containing 1 p.p.m. of fluorides results in a very mild mottling of the enamel (chalky-white spots) in about 10 per cent of the exposed persons (61) (62).

Biochemical analysis shows that the fluorides ingested during the period of development is stored in the hard tissues throughout life (6).

Growing dentin. The growing and calcifying dentin responds much less to fluorides than does the developing enamel. The calcification of the dentin becomes interglobular and stratified only after relatively large doses. Disturbances in the formation of the dentin matrix (vascular and pulpal inclusions) occur only in prolonged experiments and are probably the result of cumulative effects of fluorides (218) (230). Calcium has a protective action against fluorine intoxication and variations in the calcium and phosphorus content of the diet may modify the degree of response of the dentin (126) but fail to nullify the effect of the fluorine (237). Fluorine when added to the diet is most toxic in water, less in milk and least in mixed foods (111).

Periodontal structures. The periodontal structures show the least disturbance. In dogs, prolonged feeding of fluorides produces changes that can be recognized radiographically and that consist of an irregular and indistinct outline of the periodontal membrane and the alveolar bone trabeculae (206).

Adult teeth. Completely formed and calcified enamel and dentin are not affected by fluorides. An individual who has lived many years in an endemic area which contains sufficient fluorine in its water supply to produce mottling of the teeth in the children (usually more than 1 p.p.m.) shows no effects in his teeth provided he settled there after tooth development had been completed (181).

Fluorides and Caries. Recent experimental, biochemical, clinical and bacteriologic investigations show that mottled enamel is less liable to dental caries (64) (5). Fluorides added to the diet inhibit the rate of experimental caries progress in the rat (193) (75). However, doses up to 10 p.p.m. must be used to be effective (177). Miller (193) explains the action of fluorides as an inhibition of the phosphorylation mechanism in the carbohydrate breakdown by the bacteria. Another possible explanation is that an optimal amount of fluorine in the enamel makes it more resistant to acid dissolution (5).

It has been estimated that in man the daily ingestion of 0.5 to 1.0 mgm. of fluorides during the first eight years of life would reduce caries about 50 per cent (179) (62). This fact has led to many investigations attempting to utilize fluorides to prevent or lower the incidence of dental decay by topical application (161) or by adding fluorides to the communal water supply in minute quantities (1 p.p.m. or less) (64). The dangers of chronic fluorosis call for caution and further investigation before current proposals to add fluorine to water supplies are given general acceptance (64).

CARBOHYDRATES. Carbohydrates are the primary source of energy for the body. However, the American people tend to consume excessive amounts of this nutritional element. Restriction in the excessive consumption of highly

refined sugars in the form of sweetened beverages and candies is desirable from the standpoint of public health since they tend to displace other essential nutriments (23).

Most dental investigators agree that the ingestion of excessive amounts of fermentable carbohydrates accelerates the progress of dental decay (128) (60) (41). Reduction of carbohydrate intake, on the other hand, tends in most cases to slow down or even arrest the progress of decay (60). The Lactobacillus acidophilus count, which is a dependable indicator of caries activity, can be raised by increased ingestion of carbohydrates or lowered by a decreased intake of carbohydrates (20) (128).

The highly refined sugars are rapidly broken down by bacteria with the production of free acid metabolites (84). The ingestion of large amounts over long periods of time as is found in children who habitually suck on large quantities of hard candies or in pastry bakers results in a characteristic "sugar caries" (194). The latter tends to be of a characteristic cervical type and apparently begins through a decalcifying action upon the enamel (194).

Experimental studies suggest that an excessive intake of carbohydrates does not initiate caries but rather increases the rate of decay already present (149) (58) (166) (209) (212) (52).

Acid Foods. Although acid is produced by bacterial action incident to carbohydrate degradation (84), free acid may also be ingested with foods. The latter tends to decalcify the enamel surface rather than to produce typical caries as occurs in the carbohydrate degradation by bacteria. The local decalcifying action depends upon the concentration and amount of exposure (271) (155) (178). However, the buffering action of the saliva is usually sufficient to protect the enamel (85) (3).

The presence of citric acid in acidified candies constitutes a distinct hazard to the enamel. West and Judy (271) found that a 40 per cent solution of acidified candies in water had a pH of 2.5 to 2.8 and dissolved enamel in six hours. The manner in which children suck upon these candies over long periods of time permits a high local concentration upon the tooth surface (271) (194).

The effect of the indiscriminate use of concentrated beverage flavors and beverages containing large amounts of citric and tartaric acids (178) as well as the excessive use of lemons and lemon juice should also be considered in respect to their decalcifying action upon the enamel (155).

Acid-Base Character of the Diet. Alveolar bone. The excessive ingestion of foods with an acid reaction or acid ash results in a generalized acidosis and in the withdrawal of calcium salts (by resorption) from the spongy bone (134). The urine becomes acid in reaction. The supporting spongiosa of the alveolar bone shows a marked resorption and osteoporosis so that the teeth become loose (134) (135) (9).

Caries. The possible rôle of acidosis or alkalosis in the production or prevention of caries is not so clear. Jones (138) associated an excessive intake of acid ash foods with the presence of rampant decay. Kugelmass et al. (165) observed an arrest in the progress of decay when children were placed on an alkalizing diet.

Forshufvud (83) produced rampant caries in rats by daily disturbances in the acid-base equilibrium of the blood by means of alternate injections of 1.5 per cent ammonium chloride and 3.0 per cent sodium bicarbonate. On the other hand, Thomas and Bodecker (248) who repeated the experiment, were unable to observe any effect on the incidence of caries. Rosebury and Karshan (209) altered the acid-base balance in rats by adding citric juices and synthetic mixtures to the diet. They also found no effect on caries.

The problem of acid-base balance of the diet and acidosis or alkalosis in relation to caries needs further investigation, especially in respect to the effect upon the salivary pH and the relation between urinary and salivary pH. Forbes and Gurley (82) observed changes in the acid neutralizing power of the saliva with different diets. They found that the pH of the saliva was lowered by a high cereal or high grain (acidifying) diet and increased by a high vegetable and fruit (alkalizing) diet. The relation to caries per se has not been studied.

PHYSICAL CHARACTER OF FOOD. In contrast to the chemical or nutritional elements of the food which are assimilated and distributed through the blood stream, the physical character of the food is concerned with the topical effects upon the teeth and periodontal structures and acts only in the oral cavity. The effects may be beneficial or harmful, depending on whether the foods are detergent and stimulating in action or whether they are adhesive, retained and impacted so that they undergo local decomposition. Thus while the physical character of the food has no direct effect on the enamel and dentin growing within the bony jaw, it constitutes an important dietary (rather than nutritional) factor in caries and periodontal disease once the teeth are erupted into the oral cavity.

Physical Character of Food and Caries. Particle size and initiation of caries. Caries can be initiated in the molars of the rat by food of a particular particle size which tends to impact in pits and fissures (60-mesh screen) (114). This initiation of caries is not dependent upon the nutritional elements of the diet since the caries can be produced even with a diet otherwise completely adequate and can be prevented by merely changing the size of the food particles to a very coarse or very fine state, thus eliminating their impacting quality. These facts have repeatedly been verified (167) (149). Rosebury (207) explains the caries initiation as a result of the impaction which provides a well-protected area for bacterial action upon the carbohydrates present in the food. Rosebury and Karshan (211) were thus able to produce caries in rats by the use of Pilot biscuit which they had observed had similarly caused caries among Esquimos (213).

Detergent action. The relation between the detergent and abrasive quality of the diet and the incidence of dental caries in man has been pointed out many times (255) (256) (207) (198) (156) (60). Wallace (256) points out that chewing materially aids in keeping the mouth clean. Hard, coarse and fibrous foods which cannot be swallowed without thorough chewing produce a natural cleansing action of the tooth surface. Studies have shown that when whole citrus fruits (rather than their juices) are added to the regular diet, the bacterial count and bacterial fermentation (and therefore by implication, caries activity) are markedly

reduced (160). Howitt, Fleming and Simonton (119) and Haydak et al. (106) showed that a non-detergent or soft diet results in the rapid accumulation of epithelial and bacterial débris upon the surfaces of the teeth. Although the bacterial count is markedly increased by this type of diet, the actual effect on caries progress was not measured.

Attrition. Klatsky and Klatell (156) and Day (60) point out that in primitive peoples, on a diet which caused marked attrition of the teeth, the incidence of caries is much less than in civilized peoples on a much softer diet which requires less masticatory function.

Protective action of lipoids. Rosebury and Karshan (208) observed that while experimental caries in the rat was not prevented or cured by additions of vitamin D to the experimental diet, the rate of progress was retarded. They felt, however, that part of the protection lay in the vitamin-free corn oil. In 1939 (210) they showed that vitamin-free corn oil, Wesson oil, Crisco and lard were effective in reducing the caries index. Box (29) and E. V. McCollum et al. (180) have extended these observations to man. They believe that the lipoids in the diet may form a protective mechanical coating upon the teeth and thus prevent access to bacteria or their acids.

Thus it may be concluded that the physical character of the diet is an important consideration in the problem of dental caries.

Physical Character of Food and Periodontal Disease. Hard foods which require thorough mastication are not only detergent in action but also stimulate the periodontal structures and promote the blood supply (45) and hornification of the gingivae (197). Thorough chewing leads to intermittent stresses which are transmitted to the periodontal structure and become transformed into intermittent tension forces upon the alveolar bone. The latter responds by reorganization and apposition. Soft and adhesive foods, on the other hand, lead to local gingival inflammation (197) (45), and may serve as a nidus for calculus formation. Haydak et al. (106) fed a diet consisting exclusively of cow's milk and honey to five adult men. A heavy white cheesy film covered the teeth and gums. The gums were hyperemic and red. They explained these findings on the basis of a lack of stimulation and mechanical cleansing normally derived from the mastication of solid food. Mead (182) found that the daily addition of one and one-half grapefruit to a regular ration resulted in improvement of gingival health. It is quite possible that this result is due to the local cleansing effect of the fruit in addition to the possible benefits of increased vitamin C intake. The enamel showed no decalcification or other deleterious effects.

The evidence seems to be conclusive that foods when in a physical state which requires thorough mastication tend to promote oral hygiene and the health of the periodontal structures.

Sharp, splintery foods may be conducive to local injury when the food particles become embedded in the soft tissues and act as foreign bodies. An excessive diet of oats in rabbits, guinea-pigs, rats and mice produces widespread gingival necrosis and destruction of the supporting dental apparatus (259) (99) (274).

DIET AND DENTAL DISEASE. Any analysis of the influence of the various

nutritional factors on the diseases of the teeth must take into consideration the difference between the growing and the adult enamel and dentin. Hypocalcification (deficient calcification) and hypoplasia (deficient formation) of the enamel and dentin are of developmental origin and can therefore result only from nutritional disturbances occurring during tooth development. Dental caries, on the other hand, is an acquired post-developmental disease beginning upon the surface of the enamel and is therefore not influenced directly by nutrition but rather through factors present locally within the oral cavity. The periodontal diseases hold an intermediate position and can be influenced by both systemic and local factors.

Dental Caries. Dental decay is not a developmental defect but an acquired, post-eruptive disease which attacks only the completed intra-oral portion of the tooth. The progress of dental caries is not influenced by the mineral content of the teeth except for fluorine (4). Many careful clinical investigations disprove the view that caries can be produced or prevented by altering the intake of vitamins or minerals (57) (60). Disturbances in calcification (as mottled enamel or rickets) or in formation (as enamel hypoplasia) also do not play a significant rôle in the etiology of dental caries but at best only influence the rate of its progress.

While a balanced, adequate diet is always indicated, it is in itself no guarantee against the ravages of tooth decay (207). Caries frequently occurs in individuals who enjoy optimal nutritional intake and can be produced experimentally in spite of a properly balanced diet (51) (207). On the other hand, caries has frequently been absent in individuals who suffered from nutritional deficiencies (159) (60). The low incidence of caries among native populations in different parts of the world has not been the result of any one dietary regimen (234).

In spite of these limitations in the effectiveness of a balanced diet in combatting dental caries, there have been indications on the basis of clinical (117) (118) (30) and experimental (207) data that an adequate diet may have some influence in retarding the rate of caries progress. The mechanism of such partial protection against caries is not known. It is probably related to a modification of the saliva and the oral milieu—a factor which appears to be all-important in the etiology and progress of dental caries (29).

The local action of the food upon the tooth surface may play an essential rôle in the initiation and progress of dental caries since food stagnation and decomposition on the surface of the enamel favor bacterial activity and acid formation, especially when the fermentable carbohydrates serve as the substrate (85). Dental caries is a problem of food decomposition in the oral cavity rather than of food absorption in the gastro-intestinal tract. The physical nature of the diet may thus be more important in preventing and controlling caries than the chemical content (60).

Present status of caries research. The problem of caries is still unsolved and highly controversial (97). Experimental studies in animals have been complicated by the anatomical differences in the teeth of different species. Dog experiments have failed to produce caries (187). Continuously growing teeth in rodents do not decay since their rapid eruption and wear do not permit local food

stagnation and impaction. However, caries may occur in continuously growing teeth when retarded eruption and imperfect formation and shape permit food decomposition on the non-cleansing, imperfectly abrading surfaces (274) (77).

Clinical studies of caries for the most part have given inconclusive results because of inadequate and subjective methods of assessing caries activity and because of insufficient and inadequate controls (52).

Hypocalcification and Hypoplasia. These clinical conditions are of developmental origin. The growing enamel and dentin are selective in their response to various vitamin and mineral deficiencies. Mild fluorosis will readily produce hypocalcification of the enamel without a corresponding response in the dentin. On the other hand, subclinical deficiencies in vitamin D or calcium will result in imperfectly calcified dentin without affecting the calcification of the enamel. These hypocalcifications are not uncommon. Enamel hypoplasias, however, result from permanent cell injuries and are rarely caused by a nutritional deficiency alone. For example, enamel hypoplasia cannot be predicted merely on the basis of an early history of rickets, but may be expected when there is a concomitant parathyroprivic tetany.

Once produced, hypocalcifications and hypoplasias are permanent and cannot be corrected by any dietary regimen. It must also be pointed out that affected teeth are not more prone to decay than are sound teeth.

The view that the depth of occlusal pits and fissures (which factor plays an important rôle in caries susceptibility) can be controlled by diet is erroneous. These structural defects are genetically determined and are the result of the fusion of the appositional growth centers established during tooth development at the relatively early stage of morphodifferentiation (227).

Periodontal Diseases. The nutritional status of the patient must be considered in both the diagnosis and treatment of the periodontal diseases.

The periodontal tissues (gingivae, periodontal membrane, alveolar bone and cementum) are of continuous growth and sensitive to both systemic and local factors. Nutritional disturbances can affect their resistance and structure, as in the case of scurvy and pellagra. It must be pointed out, however, that the majority of cases are of local origin and their treatment consists of oral hygiene, local prophylaxis and the elimination of local irritants. When local treatment is ineffective, systemic factors must be considered. In apparently otherwise healthy patients who reveal no systemic disturbances, the possibility of a subclinical nutritional deficiency must be investigated and where indicated therapeutically treated.

SUMMARY AND CONCLUSIONS

The oral structures show early and characteristic changes in response to nutritional disturbances. Nutritional studies, therefore, should include gross and histologic examination of the following structures:

1. The enamel and dentin
2. The periodontal structures (gingivae, cementum, periodontal membrane and alveolar bone)

3. The tongue
4. The lips
5. The oral mucosa

The type of response varies with the particular dental or oral structure and its stage of development, with the specific nutritional disturbance, and with the species studied.

The growing and calcifying enamel and dentin serve as a kymographic biologic record of the nutritional status of the individual and are especially sensitive to calcium, vitamins A, C, and D and fluorine.

The fully formed and calcified enamel and dentin are no longer influenced by calcium metabolism: They contain the nutritional record of the past. The completed enamel is exposed to the oral fluids and oral flora and is beyond the pale of systemic nutritional influences. It is essentially devoid of an internal environment and is influenced by the latter only indirectly and to the extent that the oral milieu may be modified through systemic changes in the saliva or in the oral epithelium.

The soft oral structures—the oral mucosa, the tongue, the lips, the gingivae and the periodontal membrane—which grow throughout life reflect the nutritional status of the present in both the young and the old individual and are especially sensitive to vitamins B, A and C.

The alveolar bone, which also grows throughout life, responds particularly to disturbances in mineral metabolism and offers the advantage of radiographic examination.

Our knowledge of the effect of nutritional disturbances on the oral structures is derived from both experimental and clinical observations.

Most of the evidence on the delicate response of tooth development to nutritional disturbances is derived from animal experimentation, especially from rodents. Their incisors are of continuous growth and serve as ideal test objects for the analysis of growth, calcification and eruption.

Where clinical data are not available, it cannot be assumed that the oral structures in man are equally sensitive, either qualitatively or quantitatively. There are considerable anatomic and physiologic differences in the dental apparatus of the rat, guinea pig, dog and other species.

The findings on the response of the soft oral tissues are derived largely from clinical observations in man. This is especially true of the effects of vitamin B deficiency. The experimental and microscopic oral changes in this deficiency await further investigation.

Present evidence has not established a specific nutritional basis for caries or for periodontal disease. Dental disease may be caused or aggravated by nutritional deficiencies and in such cases cannot be treated successfully unless the nutritional condition is recognized and corrected.

The physical character of the food, through its cleansing action on the enamel surface and its stimulating action on the gingivae, is a significant dietary factor in caries and periodontal disease.

Caries is largely a problem of food decomposition in the oral cavity rather than of gastro-intestinal absorption.

The effects of proteins, fats and carbohydrates upon the teeth and surrounding tissues merit further investigation.

Routine examination of the oral structures offers an accurate index to the state of nutrition of the individual.

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THE PHYSIOLOGICAL EFFECTS OF SUNLIGHT ON MAN

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The first problem confronting the writer of a review on the physiological effects of sunlight is to set the limits of his discussion, for sunlight exerts its influence indirectly upon so many aspects of life that one might embrace a vast field under this heading. When the topic is limited to the specific effects of sunlight impinging directly on the human organism, as it will be in the present instance, the field is greatly narrowed; but even then it is necessary to touch at least briefly on some of the indirect effects. For example, the heat load contributed by sunlight falling directly upon the human body is only one part of the total heat load to which the sun also contributes indirectly, and its physiological effects cannot be studied separately.

There exists a widespread belief in the beneficial effects of sunlight, which finds expression in the current fad of sun-bathing. This belief stems in part from false analogies that have been drawn. For example, the idea that since sunlight is beneficial and necessary to plants it must be beneficial and necessary to man, which—however absurd—is found frequently in scientific literature, sometimes directly stated, sometimes implied. The detrimental effects of sunlight have received much less consideration and publicity, yet when one sets about the preparation of a critical review he is struck by the fact that the balance is weighted heavily on the side of effects which appear injurious rather than beneficial. Perhaps if some of the many claims were supported by better experimental evidence this would not be true, but if this review were restricted to a consideration of proven beneficial effects it would find a paucity of material. If, then, these pages seem to be more concerned with pathological processes than the reader may have expected, it is hoped they will not encourage him to adopt a troglodytic existence. Too much has already been written about the dire effects of tropical sunlight, and it would be unwise to revive the wearing of red spine pads or other paraphernalia designed to combat dangers that do not exist. It is hoped, however, that he may be led to recall Phaethon's misfortune at least as often as the triumphs of Mithra.

Physical Aspects

Any review of this subject must presuppose knowledge of the physical factors involved, but the specific type of information required is not always readily available. Hence a brief consideration of certain aspects of the physics of sunlight, and of other essential matters precedes the discussion of physiological effects.

The spectrum of sunlight. The maximum intensity of the solar spectrum just

[1] The opinions or assertions contained herein are the private ones of the writer, and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

outside the earth's atmosphere lies at about wavelength 0.48μ [2], which corresponds to the maximum for a black body at 6000° K, the temperature generally assigned to the outer surface of the sun. Small periodic changes in the intensity of sunlight reaching the earth's atmosphere may have important effects on climate, and hence on the behavior of mankind (e.g., 1, 144, 145) but so far as direct physiological effects are concerned, such fluctuations are unimportant, since variations in the intensity and spectral distribution of sunlight with season, latitude, and time of day, and with changes in the earth's atmosphere are so very much greater.

In passage through the atmosphere the spectrum of sunlight is modified because the atmosphere absorbs and also scatters some wavelengths to a greater extent than others. Ozone, which removes the shorter ultraviolet wavelengths, and water vapor which absorbs the longer infrared wavelengths are the principal absorbers, smoke, dust and the other gaseous constituents absorbing to a lesser extent. Scattering by gas molecules, by dust and smoke particles, and by water droplets affects the spectral distribution because short wave lengths are scattered more than long ones. Some of the scattered radiation reaches us as "sky-radiation" which, being relatively rich in the short ultraviolet wavelengths, may be a potent source of sunburn. The factors that determine the spectrum and intensity of sunlight at the earth's surface have recently been analyzed by Moon (202), who presents an approximate set of values based on the measurements of various investigators, which is useful in estimating the composition and intensity of sunlight under various conditions; the curves shown in figure 1 were drawn from his data. For values at the short wavelength end of the solar spectrum, particularly interesting physiologically, recent calculations by O'Brien (207) should be consulted.

Transmission of light by the atmosphere obeys the following equation very closely for any given wavelength, λ:

$$I_\lambda = I_{o\lambda}\, e^{-m_\lambda \sec z} \qquad (1)$$

wherein I_λ is the intensity of radiation of wavelength λ arriving at the surface of the earth, $I_{o\lambda}$ is the intensity of this radiation arriving at the outer margin of the earth's atmosphere, m_λ is dependent only on λ, and z is the angle which the sun subtends with the zenith, called the zenith angle. The length of atmosphere through which the sun's rays must pass (air mass) is directly proportional to the secant of z. When the sun is at zenith, sec $z=1$, and the sun's rays pass through the shortest possible length of atmosphere, or air mass 1. When the sun is at 60°, i.e., four hours from the zenith, sec $z=2$, and the sun's rays must pass through twice as great a length of atmosphere, i.e., air mass 2. In figure 1, curves 0, 1, and 2 represent solar spectra for these three air masses respectively. The curve for air mass 0, i.e., outside the earth's atmosphere, is obtained by extrapolation from measurements made at different zenith angles.

[2] Because of the wide range of wavelengths discussed herein, it will be convenient to use the micron (μ) as the unit of wavelength rather than the more customary units, the millimicron (mμ) and the Angstrom unit (Å). $1\mu = 10^3$ mμ $= 10^4$ Å.

The coefficient m_λ varies with the wavelength according to the extent of absorption and scattering by the atmospheric constituents, but remains approximately constant for a given wavelength. It is obvious from equation (1) that the magnitude of m_λ determines the extent to which the intensity I_λ varies with zenith angle, and hence with latitude, season and time of day; for a wavelength for which m_λ is large, the variation with zenith angle is greater than for a wavelength for which m_λ is small. Thus, for example, m_λ is very large for the sunburn producing wavelengths in the neighborhood of 0.3μ due to the fact that

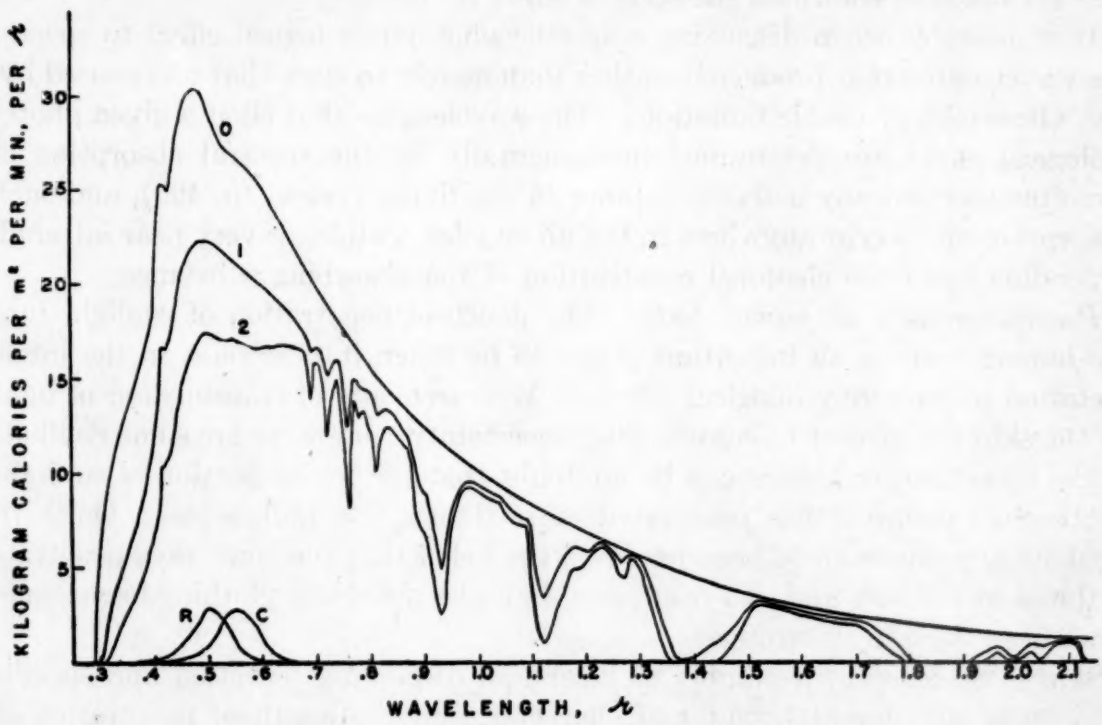

Fig. 1. Spectral distribution of sunlight; *0*, outside the atmosphere (air mass 0); *1*, with the sun at zenith (air mass 1); *2*, with the sun at 60° from zenith (air mass 2). Curves 1 and 2 are for 20 mm. H_2O, 2.8 mm. ozone, and 300 dust particles per cm^3. From the data of Moon (202).

Curves *R* and *C* indicate respectively, the sensitivity of the human rods and cones; the ordinate units for these curves are arbitrarily chosen.

atmospheric ozone absorbs these wavelengths strongly, and hence the intensity of these wavelengths in sunlight varies widely with time of day. On the other hand, m_λ for the principal wavelengths perceived by the eye is relatively small since there is little absorption by the atmosphere in this spectral region. Thus, after five o'clock on a midsummer afternoon in low latitudes one is not likely to be sunburned, although the sun is still shining brightly insofar as the eye can determine. The deep indentations in the infrared region of curves 1 and 2 in figure 1, represent high values of m_λ, and correspond to absorption by water vapor. Obviously the infrared portion of sunlight varies more with zenith angle and with the quantity of water vapor than does the visible part.

The division of sunlight into ultraviolet, visible and infrared spectral regions is arbitrary, but useful. Light, or visible radiation, is usually defined as that

radiation perceived by the human eye, and is often said to lie between 0.4μ and 0.7μ, the approximate limits of photopic vision; radiation of shorter wavelengths is referred to as ultraviolet, that of longer wavelengths as infrared. The arbitrariness of this classification is seen by reference to curves *C* and *R* in figure 1, which describe, respectively, the spectral sensitivity for photopic, and for scotopic vision; for any limits assigned to these curves cannot be exact because the sensitivity falls off very gradually as the curves approach zero. Actually the rods perceive radiation of wavelengths as short as 0.3μ, but the sensitivity is so slight that it cannot be shown on the scale of curve *R* (106).

It is desirable when discussing a specific photophysiological effect to specify the wavelengths that produce it, rather than merely to state that it is caused by, say, ultraviolet or visible radiation. The wavelengths that elicit a given photobiological effect are determined fundamentally by the spectral absorption of some photochemically active substance in the living system (p. 490), and such absorption may occur anywhere in the ultraviolet, visible, or very near infrared, depending upon the chemical constitution of the absorbing substance.

Penetration into the human body. The depth of penetration of sunlight into the human body is an important factor to be taken into account in the interpretation of photophysiological effects. Measurements of transmission of light by the skin are subject to considerable uncertainty, and there are some conflicts in the literature; but there can be no doubt that the major portion of sunlight is absorbed before it has penetrated more than a few millimeters. Quite ridiculous hypotheses have been based on the belief that the sun's rays penetrate to the deeper tissues and as a result certain useles articles of clothing have sometimes been worn in the tropics[3].

Skin is made up of a number of layers, all displaying reflection and specific absorption of different extent and character, and the depth of penetration of radiation depends upon a number of factors, the relative importance of which varies with the wavelength of the radiation. Consideration of the fate of beams of monochromatic radiation of a few selected wavelengths impinging upon the skin will serve to illustrate this.

A beam of radiation of wavelength 0.28μ is scattered in all directions before it has penetrated far into the corneum, due to the tiny flake-like elements composing this layer, which represent the remains of the dead epidermal cells. Reflection and refraction at the boundaries of optical discontinuity presented by these elements renders the corneum an effective diffusing medium. The protein of the corneum strongly absorbs this wavelength, with the result that only a small fraction of the incident beam is reflected back from the skin (176, 239). Absorption is greatly enhanced by diffusion since that radiation which is scattered laterally must pass through a longer path of absorbing material than that passing through normal to the surface. This is shown by the great increase in transmission that results when the corneum is infiltrated with a "clearing agent" of appropriate refractive index, which fills up the space between the elements and reduces the reflection and refraction at their boundaries (175), and by measure-

[3] e.g., orange-red underwear and helmet linings (219), and red spine pads (29, p. 280).

ment of the light diffused at wide angles (155). The attenuation of radiation by such a system may be approximately described by the following equation:

$$I_\lambda = I_{o\lambda}\, e^{-k(a_\lambda, s_\lambda)l}. \qquad (2)$$

in which $I_{o\lambda}$ is the intensity of the incident radiation of wavelength λ, I_λ is the intensity of such radiation after passing through thickness l of the absorbing medium, and $k(a_\lambda, s_\lambda)$ is the attenuation coefficient. The absorption function a_λ, and the scattering function s_λ, are mutually dependent, but vary to different extents with different characteristics of the attenuating medium, in this instance the various layers of the skin. For example, after passing through the corneum to the living layers of the epidermis, the scattering of wavelength 0.28μ undoubtedly diminishes because the optical discontinuity at the cell boundaries is not as pronounced as at the boundaries of the elements of the corneum (54). Thus the equation must be applied with caution, but is useful in a general way; for example, it serves to remind one that the intensity falls off approximately exponentially as the depth of penetration increases, and that any estimation of maximum depth of penetration can mean only the depth at which some small fraction of the incident energy is still detectable. In the case of wavelength 0.28μ the intensity is greatly reduced in passing through the corneum, and virtually none of it passes the epidermis to reach the most superficial blood vessels in the papillary layer of the corium. Very little radiation of this wavelength is reflected back from the skin surface. The functions a_λ and s_λ also vary widely with wavelength.

Radiation of wavelength 0.5μ also diffuses upon entering the corneum, but is relatively little absorbed. Hence a large fraction is reflected back from the skin (48, 49, 84, 238), while another large fraction passes through the corneum and the living layers of the epidermis to the blood vessels in the corneum (9, 15, 212). Here the radiation is absorbed strongly by the hemoglobin of the blood. The effectiveness of the corneum in scattering such radiation is demonstrated by the fact that the capillaries themselves may be rendered visible through a microscope if scattering is reduced in the corneum by a "clearing agent" such as immersion oil. Since the absorption by the overlying layers is not great, some of the radiation diffusely reflected from the skin comes from the deeper layers in which the blood vessels are situated. Thus, under ordinary illumination the color of the skin is influenced by the amount and degree of oxygenation of the blood in the superficial vessels, because some wavelengths are absorbed to a greater extent than others.

At wavelength 0.6μ much the same situation exists except that there is little absorption by the hemoglobin, and considerable energy of this wavelength may reach the subcutaneous tissues (9, 15, 212).

Wavelength 3.0μ like wavelength 0.28μ is largely absorbed by the corneum. Scattering is much less pronounced at this wavelength (117, 118, 213).

None of the numerous studies of skin transmission that have been made, presents a complete quantitative picture covering the ultraviolet, visible and infrared. An attempt to fit together such a picture would entail a consideration

of the relative accuracy of the methods used by the various investigators, and it is doubtful that this would accomplish much beyond revealing a number of conflicts. A general summary of the clearly established facts may be helpful, however. Transmission is very low for wavelengths shorter than 0.32μ, a spectral region of particular interest biologically. Such radiation is largely absorbed in the epidermis, principally by proteins (175). Measurements have been made by a number of investigators (9, 155, 175, 212), which agree well for these and longer wavelengths in the ultraviolet. Anderson and Macht's (8) measurements have been frequently quoted as indicating much greater transmission, but Anderson and Fraser (7) using the same method later obtained values in line with those cited above. Bachem and Kuntz' (14) values seem somewhat high, whereas earlier estimates (122, 124) are too low because the methods used neglected much of the scattered radiation. Transmission increases progressively with wavelength through the near ultraviolet, and is high throughout the visible, considerable amounts of visible radiation reaching the corneum and even the subcutaneous tissue. Transmission remains high in the near infrared, but deep indentations appear in the curve at wavelengths of maximum absorption by water, and at the long wavelength limit of sunlight (about 3.0μ), penetration is very slight (118). There is considerable disagreement as to maximum values for penetration, which various investigators place at from 0.7μ to 1.5μ (15, 53, 105, 138, 211, 212). The temperature of the tissues at some depth may be raised appreciably when radiation impinges on the skin surface (163), but this does not indicate actual penetration of the incident radiation, and Hardy and Muschenheim (117, 118) believe that the high transmission values obtained by some investigators result from the inclusion of radiation re-emitted from tissue heated in this way. The latter investigators found the maximum infrared transmission of human skin 2 mm. thick to be about 6 per cent, whereas Cartwright's measurements (53) (also cited by Danforth, 69) indicated that over 20 per cent of radiation of wavelength 1.15μ passed through the human cheek, a thickness of 5 mm. Even if the highest values obtained could be accepted, it is not conceivable that an important fraction of sunlight reaches skin-tissues more than a few millimeters beneath the surface. The tremendous penetrations reported by Baldery and Ewald (18) are so out of line with any other findings that one is forced to conclude that faulty technique was employed. Those wavelengths that produce the most striking effect in human skin, sunburn, penetrate only a small fraction of a millimeter.

Little scattering takes place in the eye as compared to the skin, and consequently penetration is much deeper. The ocular media are very transparent to some wavelengths, but there is considerable selective absorption of others. There are no quantitative data for transmission in the ultraviolet, but it may be estimated from semi-quantitative measurements (81) that wavelengths shorter than 0.32μ are largely absorbed in the corneum and conjunctiva, although there is some penetration as far as the epithelium of the lens (251); this low transmission is no doubt due, in the main part, to absorption by proteins. Ludvigh and McCarthy's (179) measurements show only 9 per cent transmission at 0.4μ

for the total eye media exclusive of the retina, but the transmission rises rapidly throughout the visible to reach a maximum at about 0.8μ. In the infrared we have the data of Hartridge and Hill (121) and of Roggenbau and Wetthauer (224) for the eye of the ox, and from these, estimates for the human eye may be made by correcting for the differences in thickness of the various layers. When this is done it is found that transmission to the retina falls to zero at about 1.4μ. This is approximately the same limit that would be set by a layer of water of the same thickness as the eye, and it is probable that this substance is the principal absorber of the infrared radiation of sunlight (121). Virtually all of the radiation that penetrates to the retina is absorbed by that structure, principally in the pigment epithelium.

TABLE 1

Reflection of total sunlight by human skin

TYPE OF SKIN	REFLECTION	REFERENCE
	per cent	
Fine white	45	(183)
Average blond	43	(183)
Dark brunet	35	(183)
White	30	(209)
Dark, partly negro	30	(183)
Hindu	22	(183)
Negro	16	(183)
Negro (I)	17	(209)
Negro (II)	19	(209)

Reflection and emission. A large fraction of total sunlight is reflected by human skin as is shown in table 1, which includes a number of measurements on different types of skin. It will be noted that the percentage reflected varies considerably for different complexions, but that less than half is reflected by the fairest skin. For detailed studies of spectral reflection the following references may be consulted: 48, 49, 84, 117, 118, 176, 242, 238, 239.

There is virtually no reflection of wavelengths longer than those found in sunlight, the total radiation being absorbed very superficially. The human body, like any other body having a temperature in the neighborhood of 37°C. emits a broad band of wavelengths with a maximum at about 10μ. In this spectral region human skin behaves virtually as a perfect radiator, or black body (116). This emitted radiation, which must be clearly distinguished from reflected sunlight, is often referred to as "black body radiation".

Physiological Effects

Direct action of sunlight on the human body must be limited to superficial organs, the skin and the eye. In this review these direct, superficial effects will be considered first; after which phenomena resulting indirectly from them, which involve the deeper organs of the body, will be treated.

The direct effects themselves are of two kinds, a clear distinction beween which should be made, (a) specific effects initiated by a photochemical reaction, and (b) non-specific or "radiant heat" effects, which merely result from a local rise in temperature. The former result from the activation of molecules by the capture of quanta of radiation, such capture constituting the primary act in a photochemical reaction. The type of chemical reaction which follows this primary act is determined by the kind of molecules present in the environing system as well as by the activated molecule itself (29). Such an effect is characterized by a specific *action spectrum*, that is, it is produced only by certain wavelengths that are specifically absorbed by the light absorber, i.e., the compound whose molecules are activated as the primary event in the underlying photochemical reaction. The action spectrum may follow the absorption spectrum so closely that the absorbing compound can be identified with considerable assurance, but the agreement is often masked by other factors (29, 36).

"Radiant heat" effects also result from the capture of quanta of radiant energy by molecules, but such capture is not followed by chemical reaction in this case. Instead, the energy of the absorbed quanta is distributed among the molecules of the system in such a way as to increase the average kinetic energy, and hence the temperature of the system. Whether a photochemical reaction occurs or not depends upon the type of molecule that captures the quantum, and also upon whether other appropriate molecules are present to participate in a photochemical reaction. If such conditions do not exist the absorbed energy will go toward heating the system; this is the fate of most of the energy of sunlight that enters the human body. Although the term is often employed, no specific "heat rays" exist, in the sense of the specific wavelengths that produce photochemical reactions in the eye or in the skin.

Effects on the skin. Sunburn. Exposure of the skin to bright summer sunlight for half an hour or longer is followed by dilatation of the minute vessels of the exposed area, manifested grossly as erythema. The erythema is accompanied by swelling, often so slight as to be almost imperceptible. If the exposure is prolonged, marked edema, desquamation or blistering may follow, and there may be pain or itching. The erythema fades in the course of a few days, being gradually replaced by "suntan" due to rearrangement and increase of melanin in the epidermis. The suntan may persist for months or even years. In addition the suntan may darken by a process beginning almost immediately upon exposure, and ceasing with the exposure. All these events have a common origin —immediate or remote—in the action of ultraviolet radiation on the epidermis, and may be logically included under the term *sunburn*, even when the observed response is minimal and may not seem to merit classification as a "burn".

The writer's concept of sunburn postulates that direct injury to the cells of the epidermis by ultraviolet radiation is the crucial mechanism underlying the events enumerated above, and that as a result of such injury the cells elaborate (i.e., produce and release) various substances which bring about specific physiological responses. The long latent period between exposure and the physiological responses may be accounted for by assuming that the physiologically active

substances are elaborated by the injured cells at relatively slow rates. Certain hypotheses based on the direct photochemical formation of physiologically active substances find difficulty in explaining the long latent period and duration of the physiological responses (p. 497). A scheme designed to represent the various events of sunburn and the processes underlying them appears in figure 2. Some of the steps in this scheme are much better substantiated by experimental evidence than others, and the pages immediately following will be devoted to an evaluation of this evidence. Various aspects of sunburn will be discussed, for convenience, under separate headings; this treatment is not intended to imply that they are separable as regards basic underlying mechanism.

The basic process. Ultraviolet radiation produces injurious effects in living systems in general, indicating that this agent acts upon some component of all

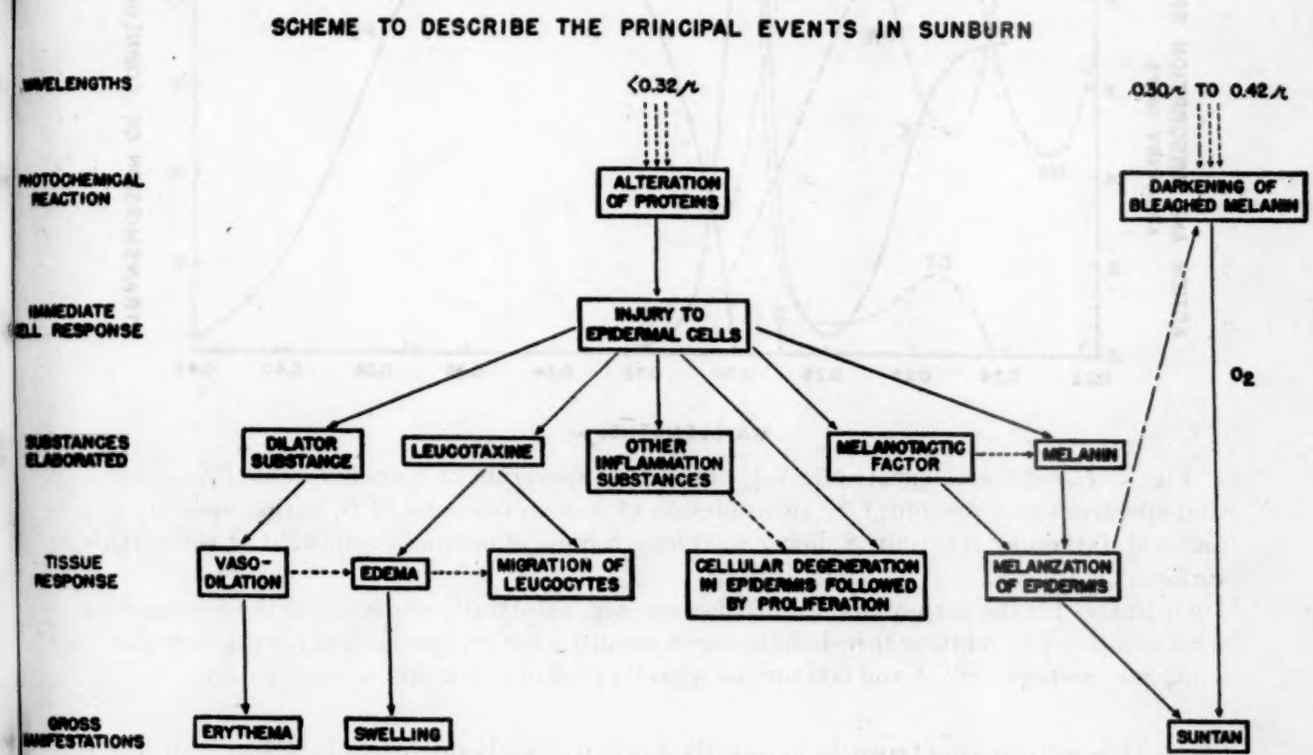

Fig. 2. A tentative scheme to describe the events of sunburn.

cells—a concept strengthened by the fact that, with very few exceptions, such effects have their long wavelength limits at about 0.32μ, and have similar action spectra. Systems comprised of single cells such as bacteria, protozoa, the sperm and eggs of invertebrate animals, etc., lend themselves to the study of action spectra for injurious and lethal effects, many of which have been determined on such systems. All these action spectra show remarkable similarity to the absorption spectra of typical unconjugated protein or of nucleic acid (fig. 3). The universal importance of these two substances, their presence in quantity in all cells, and the fact that both are photolabile, leads to the conclusion that ultraviolet radiation exerts its injurious action by altering either or both (see 31 for discussion and references). It appears that proteins may be principally

concerned in some instances, nucleic acid in others, there being some evidence that in cells with considerable cytoplasm to absorb the ultraviolet radiation protein is the principal substance attacked.

The first action spectrum for the erythema of human skin was obtained by Hausser and Vahle in 1922 (125)[4]. This was revised by Hausser in 1928 (124), and determinations by Lukiesh, Holladay and Taylor (176), and by Coblentz, Stair and Hogue (63) appeared shortly after. All these measurements are in essential agreement, and a standard curve for the erythema spectrum, by which

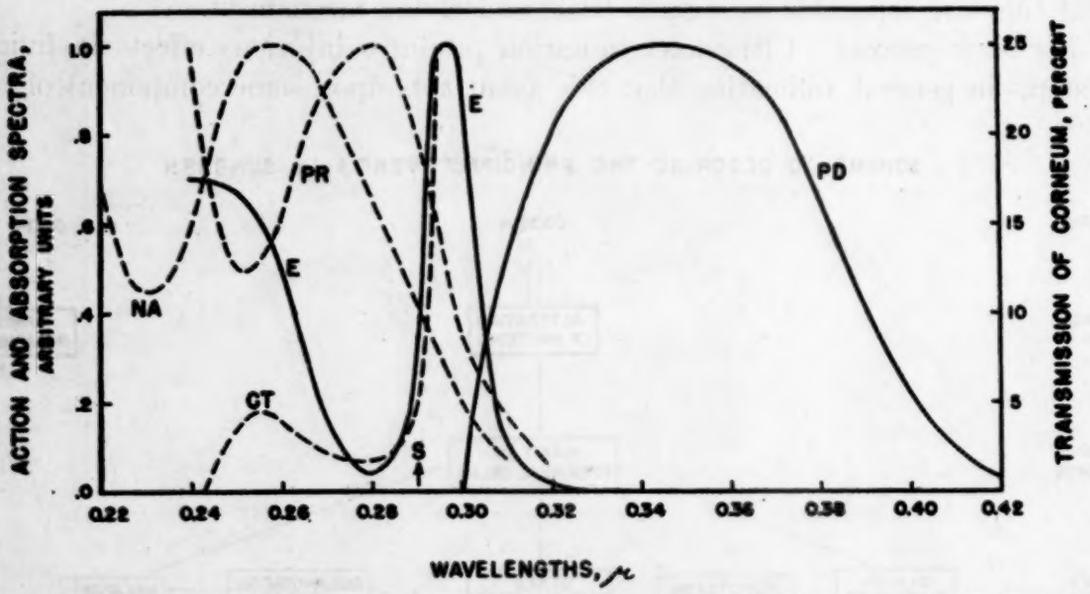

Fig. 3. *E*, erythema spectrum; *NA*, absorption spectrum of a nucleic acid; *PR*, absorption spectrum of a protein; *CT*, transmission of human corneum; *PD*, action spectrum of pigment darkening reaction; *S*, lower wavelength limit of maximum sunlight at the earth's surface.

Ordinates for the action, and absorption spectra, arbitrarily chosen to make the maxima correspond. The relative threshold energies required for erythema, and for pigment darkening are, respectively, 1 and 600 for the wavelengths of maximum action (see 36).

name this action spectrum is generally known, has been formulated by Coblentz and Stair (61).

The erythema spectrum is represented in figure 3, where it may be compared with absorption spectra of a typical protein and of nucleic acid. General

[4] The first clear recognition that sunburn is not caused by heat, but by an invisible portion of the sun's rays, seem to have come with the accidental finding that the same phenomenon results from exposure to the radiation emitted by electric arcs. An account by Charcot (55) states that the physicists Foucault and Despretz had made this discovery sometime previous to 1858, and that Foucault correctly attributed the effects to ultraviolet radiation. Only in 1889, however, was there clear-cut experimental demonstration, when Widmark (255, 256) showed that the rays of the carbon arc passing through quartz produced sunburn, whereas those rays passing through glass did not. Since glass cuts out much of the ultraviolet radiation which is passed by quartz, this experiment set the long wavelength limit for sunburn somewhere in the ultraviolet, and showed that visible radiation is incapable of eliciting the response. Although this finding was soon confirmed, no more precise determination of the action spectrum was made until 1914, when Henri and Moycho (130) carried out such measurements for the erythema of rabbits' ears.

similarity is apparent in that all three spectra have approximately the same long wavelength limit near 0.32μ, and that each has a discrete maximum, but the erythema maximum at 0.297μ does not agree with either the protein absorption maximum at 0.28μ or the nucleic acid absorption maximum at 0.26μ. Since the erythema spectrum, is measured at the skin's surface, however, it is not a correct index of the photochemical reaction underlying erythema, because this reaction takes place in the living layers of the epidermis, beneath the dead corneum which acts as a semi-opaque screen by absorbing the active wavelengths (pp. 486–488). A transmission spectrum for corneum, presented in figure 3, shows that this layer has a minimum of absorption at about 0.28μ (indicating its essentially protein character), which corresponds to the deep minimum in the erythema spectrum at that wavelength. It was Hausser (124) who originally pointed out that this maximum of absorption by the corneum accounts for the minimum in the erythema spectrum.

Taking into account the absorption by the corneum, and making certain reasonable estimates, Mitchell (201) finds that the shape of the erythema spectrum can be explained on the assumption that protein in the malpighian layer of the epidermis is the light absorber for the photochemical reaction that underlies sunburn; his is the most complete analysis of the problem that has been presented. Hamperl, Henschke and Schultze (114), on the other hand, account for the erythema spectrum in terms of the absorption spectrum of nucleic acid, and suggest that this substance is the light absorber. They state that they have found maxima in the erythema spectrum at 0.295μ and 0.26μ with a minimum at 0.275μ, and for some skins with very thin corneum another maximum at 0.23μ, but unfortunately their analysis is described rather incompletely in a brief note, which makes its evaluation difficult. In support of their thesis, these investigators point out that the nuclei of the more superficial cells of the epidermis are the first to show degeneration, and suggest that this occurs because these cells receive more ultraviolet radiation than those more deeply placed; this may not be a cogent argument.

At this point there should be mentioned some uncertainties inherent in the erythema spectrum, which render the identification of the light absorber relatively inexact. The erythema spectrum is based essentially upon the minimal perceptible erythemal dose, i.e., the minimum amount of ultraviolet radiation which elicits just perceptible reddening of the skin. But the rate of development and fading of erythema is different for different individuals (Schall and Alius, 233); hence the minimum perceptible erythema depends upon the subject chosen as well as on the time of observation, and is not an exact index of the underlying photochemical process. Hausser (124) has shown, moreover, that the relative rate of development of erythema is different for different wavelengths. The expectation that action spectra and absorption spectra will agree is based on the assumption of the reciprocity law, *intensity* $\times$ *time* = *a constant*, which does not hold for the erythema threshold at low intensities. The measurements of different investigators are in general agreement, but the amount of variation in detail (63) suggests individual variation which might indeed be expected to occur because of differences in transmission by the corneum (see also pp. 486–488).

When the relative inadequacy of measurements of absorption by the layers of the epidermis is taken into consideration in addition to the sources of uncertainty already mentioned, it becomes obvious that any attempt to identify the light absorber for sunburn can be only tentative. No definite choice can be made between protein and nucleic acid on the basis of this evidence alone; and there is room for even further divergence of opinion in identifying the light absorber, other possibilities having been suggested in connection with hypotheses which will be discussed later in this review.

Relatively small amounts of ultraviolet radiation cause physical changes in proteins in vitro (10, 72), and it is not improbable that these provide the basis for injury to the cell. But nucleic acid is also altered physically by small doses of ultraviolet radiation (141), and this might also be the basis for cell injury. The erythema of sunburn is not inhibited by depriving the skin of O_2 during the period of irradiation (44, 131), a fact that serves further to identify this process with the action of ultraviolet radiation on other organisms and biological systems; some of these systems are essentially protein and do not contain nucleic acid (29, pp. 114–117).

Considered as a whole the evidence suggests that protein is the light absorber in the sunburn mechanism, and it is credited with that rôle in the scheme presented in figure 2. Whether protein or nucleic acid or both act as light absorbers makes no difference insofar as the subsequent steps in the scheme are concerned, once the postulate is accepted that ultraviolet radiation causes injury to epidermal cells.

Such injury is indicated by the histological changes accompanying sunburn, which have been the subject of a number of studies, among which those of Keller (151), Miescher (196), and Hamperl, Henschke and Schultze (115) may be cited. There are points of disagreement, due largely no doubt to differences in the dosage of ultraviolet radiation and in the time at which the biopsies were made, but on the whole the same general picture is presented by all these studies. In no case have histological changes been observed prior to the appearance of gross erythema, at which time enlargement and engorgement of the capillaries becomes marked; intracellular edema, and the migration of leucocytes into the epidermis and into the corium in the region of the capillaries appear about the same time or somewhat later. As early as 24 hours after the exposure degenerative changes in the prickle cells are detectable; these progress and may eventually involve all of this layer. The basal cells are less affected as a rule, but may also show degenerative changes; repair usually takes place through proliferation of cells of this layer. When the acute stage is passed all layers of the epidermis with the exception of the basal-cell layer, but including the corneum, are usually left thickened. Miescher found that with large doses, endothelial cells and fibroblasts of the corium, as well as epidermal elements, show degeneration. Changes involving melanin pigment occur, which will be discussed later.

Erythema and inflammation. The appearance of alterations in the vessels of the papillary layer before changes are observed in the epidermal cells, might seem to indicate that the primary action of the ultraviolet radiation is on the

former tissues. There is strong evidence to the contrary, however. The fraction of sunburn producing ultraviolet radiation that reaches the papillary layer is small for even the most penetrating wavelengths, and some wavelengths quite effective in producing erythema virtually do not get through the epidermis. The long latent period which usually intervenes between exposure and vasodilatation, and the lack of degenerative changes in the papillary layer, except with severe dosages, support the concept that the site of injury is chiefly in the malpighian layer. Hence it is reasonable to assume that the vasodilatation results, in the main at least, from the elaboration of a dilator substance by the injured cells of the epidermis, which diffuses thence to the minute vessels of the papillary layer where it exerts its effect. This sequence of events is indicated in the above scheme. Increased concentration of dilator substance has actually been demonstrated in the skin (92, 150, 205), and in the blood of animals that have been exposed to ultraviolet radiation (157, 164).

The identification of the dilator substance released as a result of exposure to ultraviolet radiation has been the subject of considerable speculation. Lewis and Zotterman (169) suggested that it is histamine or a histamine-like "H" substance—a view that has been widely accepted. In recent years evidence has accumulated to indicate that histamine is not the dilator substance involved in various responses that resemble the histamine wheal much more closely than does the erythema of sunburn (3, 110, 189, 230), and the term "H" substance, as used to denote a substance pharmacologically similar to histamine, seems to have lost much of its usefulness and popularity. Specific differences between the effect of ultraviolet radiation on the skin and the histamine response have been shown (216), and Krogh (159) found it necessary to postulate at least two dilator substances to account for the behavior of the erythema of sunburn. Thus, although the elaboration of dilator substance seems to be the immediate cause of the erythema of sunburn, no definite statement can be made regarding the chemical nature of this substance.

Essentially the same tissue responses occur in sunburn that occur in inflammatory processes in general, and it seems not unlikely that the same underlying mechanisms operate in bringing about these responses. Menkin (190, 192, 193, 194) has recently isolated from inflammatory exudates several substances which act specifically to bring about certain of these tissue responses, and it seems probable that the complicated picture which inflammation presents may ultimately be explained in terms of such "inflammation-substances". This general thesis has been followed in introducing such substances into the scheme for sunburn outlined in figure 2. Menkin (189) has brought forth evidence that the increase in capillary permeability which occurs in inflammation is not due to histamine, as supposed by Lewis and others, but to a substance which he calls "leucotaxine". This substance also exerts a "chemotactic" effect, and by virtue of both these actions brings about the migration of the leucocytes from the capillaries into the surrounding tissues. In the scheme, leucotaxine has been included as a substance elaborated as the result of cell injury, and is credited with bringing about both the migration of leucocytes and tissue edema, the latter

being a direct result of increased capillary permeability. Indicated by a dotted line is the probable participation of vasodilatation as a lesser factor in the edema. A blanket caption has been used in the scheme to include other inflammation-substances. A substance, "necrosin", which Menkin (192) finds to cause the degeneration of cells, may be responsible in part for such changes in the epidermis and in the corium, but since degeneration of epidermal cells may also be expected to follow as a direct result of injury by ultraviolet radiation, both possibilities are indicated in the scheme. The possibility that inflammation substances exert systemic effects will be discussed later (pp. 517–518). It should be emphasized that none of these mediating substances has been isolated from sunburned skin, with the possible exception of the dilator substance; and that the scheme has not been introduced with the expectation that all its steps will be demonstrated experimentally, but to give a better picture of the complex set of processes which constitutes sunburn.

A number of other changes, not indicated in the scheme, are associated with sunburned skin; the surface temperature is raised (163, 173), evaporation of water is affected (173, 186), there are changes in electrical potential (154, 248), there is an increase in permeability of lymphatics (143), hyperesthesia occurs (240), and there are changes in chemistry including enzyme activity (2, 149, 150, 259, 260). It is probable that all these changes are inter-related with the local inflammatory process, and need no special consideration. Changes in melanin pigment will be discussed below under a separate heading.

The various wavelengths of the sunburn producing radiation penetrate to different depths (note curve *CT* in fig. 3), and it is possible that they exert somewhat different effects because they reach different layers of the skin. For example, measurements of the transmission of whole epidermis (155, 175) show that wavelengths shorter than 0.28μ do not penetrate below the epidermis, but wavelengths as long as 0.31μ may reach the corium, perhaps, in sufficient quantity to exert an effect at that level. Hausser (124) has shown quantitative differences in the effect of different wavelengths which suggest a relationship to depth of penetration. Those histological studies that have been made provide no answer to this question since all have employed spectrally in homogeneous radiation, the effects of short and long wavelengths being thus included in the same picture. It is possible that the changes in the corium which Miescher (196) and Keller (151) describe are due to direct action of the longer wavelengths of the erythema spectrum which may penetrate to these layers, but on the other hand, they may be due to a cell-injury substance (192) elaborated in the more superficial cells. That still more deeply penetrating wavelengths, longer than those of the erythema spectrum, are not responsible for any of the histological changes described above, is shown by the studies of Hamperl, Henschke and Schultze (115). Although the evidence indicates that the major part of the vasodilatation— probably all that produced by the shorter wavelengths—is due to the action of a dilator substance elaborated in the epidermis, a direct effect of longer wavelengths of the erythema producing spectrum on the vessels of the papillae cannot be completely ruled out.

Several theories for the erythema mechanism have been proposed which postulate that a dilator substance is formed directly by the action of ultraviolet radiation on components of the cells. Ellinger (90, 91, 92, 93, 94) and Frankenburger (102) suggested that the radiation acts directly upon histidine, presumably in polypeptide chains, with the result that histamine is formed. There are several objections to this hypothesis: While a dilator substance, presumably histamine (but see 249, and 29 p. 182) is formed in vitro by the action of ultraviolet radiation on histidine, the wavelengths absorbed by histidine do not correspond with the erythema spectrum (45, 94). If histamine is formed by direct action of the ultraviolet radiation the long latent period before erythema appears can only be accounted for by a slow diffusion process (93, and see 29 p. 182) and a molecule as small as histamine might be expected to diffuse much too rapidly. Moreover, the high temperature coefficient of the latent period (see p. 504) hardly seems characteristic of diffusion of such a small molecule. The long duration of the erythema, which may be present for several days after exposure, would demand that histamine continue to be formed long after the initial photochemical reaction is completed. Objections to the concept that histamine is the dilator substance in sunburn have been cited above.

Mitchell's (201) analysis of the erythema spectrum, showing that protein may well be the light absorber, has already been discussed. He suggested that the dilator substance is a breakdown product of protein, formed by the direct action of ultraviolet radiation on protein molecules; a concept more acceptable than the histamine hypothesis, since high molecular weight breakdown-products of protein might diffuse more slowly than histamine, and the process might be expected to have a higher temperature coefficient. The long duration of the erythema, however, indicates that the dilator substance continues to reach the vessels for several days after the exposure, and it seems unlikely that a simple diffusion process would continue for such a long period.

Rothman and Rubin (229) suggest that para-amino-benzoic acid is the light absorber, but have not made a thorough analysis of the agreement of the absorption spectrum of this substance with the erythema spectrum. They found that when solutions of this compound which had been exposed to ultraviolet radiation were injected intradermally, erythema of the local area developed after an appropriate latent period. These changes did not occur in the absence of molecular oxygen, whereas deprivation of O_2 during the period of exposure to ultraviolet radiation does not inhibit the subsequent development of erythema (44, 131). Another objection pointed out by the proponents of this hypothesis themselves, is that the injection of the irradiated para-aminobenzoic acid solution causes erythema but no subsequent pigmentation, whereas these two events are virtually inseparable sequelae to the exposure of normal skin to ultraviolet radiation.

Suntan. As the erythema fades it is replaced by suntan, the transition from the one state to the other being almost imperceptible. Quantitative studies of the spectral distribution of the radiation reflected from tanned and from unexposed skin indicate that their difference in color is due principally to the amount and position of the melanin pigment in the former (84, 85). In normal untanned

skin the melanin pigment is located chiefly in the cells of the basal-cell layer. A few days after exposure to ultraviolet radiation—about the time suntan first makes its appearance—the pigment begins to migrate into the more superficial epidermal layers, eventually reaching the corneum. As a result the basal cells may appear after some days to be almost free of melanin. The movement of melanin toward the surface causes the skin to present a darker appearance to the eye, even though the total amount of melanin is not increased at this early stage of suntan. Elaboration of new melanin is a later event, which is accomplished chiefly in the region of the basal cell layer (115, 151, 180). According to some investigators (e.g., 20) special dendritic melanoblasts form the melanin which they transfer to the basal cells, but according to Bloch (25, 26) and Peck (214) the dendritic cells are epidermal elements which share with the basal cells the ability to form pigment.

The migration of melanin into the superficial layers of the epidermis in sunburn (Pigmentverschiebung) reported by Keller (151) has also been noted by Peck (214) in pigmentation brought about by Thorium X (Pseudohyperpigmentation); it is suggested in the earlier observations of Lutz (180). This event occurs too early to be ascribed to epidermal hyperplasia. To account for this movement of melanin a "melanotactic factor" has been introduced into the scheme of figure 2, but without intending to assign a mechanism. Elaboration of melanin is also indicated, to represent the subsequent activity of the melanoblasts. It seems possible that the latter process is stimulated by the former, and this is suggested by a dotted line in the scheme. Both processes are represented as contributing to *melanization* of the epidermis—a term which will be used herein to include both migration and formation of melanin—and as having their origin in the injury to epidermal cells by ultraviolet radiation.

Raper, who with his collaborators has contributed much to our knowledge of the chemistry of melanin gives a series of reactions for the formation of this compound from tyrosine (see fig. 4). The first step is the oxidation of tyrosine to 3:4-dihydroxyphenylalanine, commonly known as dopa. This reaction takes place relatively slowly, and requires the participation of a catalyst, the enzyme tyrosinase, which has been isolated from numerous plants and lower animals but has not been shown definitely to exist in mammalian skin. In 1937 Arnow (11) found that ultraviolet radiation can replace the enzyme in this first step of the reaction, to bring about the oxidation of tyrosine to dopa. The exposures to ultraviolet radiation which were required were, however, much greater than those that produce suntan in human skin; but Rothman (228) found that the reaction is accelerated by ferrous salts, so that the amount of ultraviolet radiant energy required is comparable to the amount needed to produce suntan.

The concept that the initiation of the first step in Raper's reaction scheme is accomplished in the skin by the action of ultraviolet radiation meets a number of objections when the attempt is made to relate it to the events of suntan: Melanin appears as a sequel to the action of diverse agents injurious to the skin, for example, heat, friction, photodynamic action, alpha, beta and gamma

radiation, and wounds, as well as sunburn, and it is not likely that the same set of chemical reactions would be brought about directly by all these diverse agents. According to the hypothesis suggested by Arnow (11) and Rothman (228), the long latent period between exposure to ultraviolet radiation and the appearance

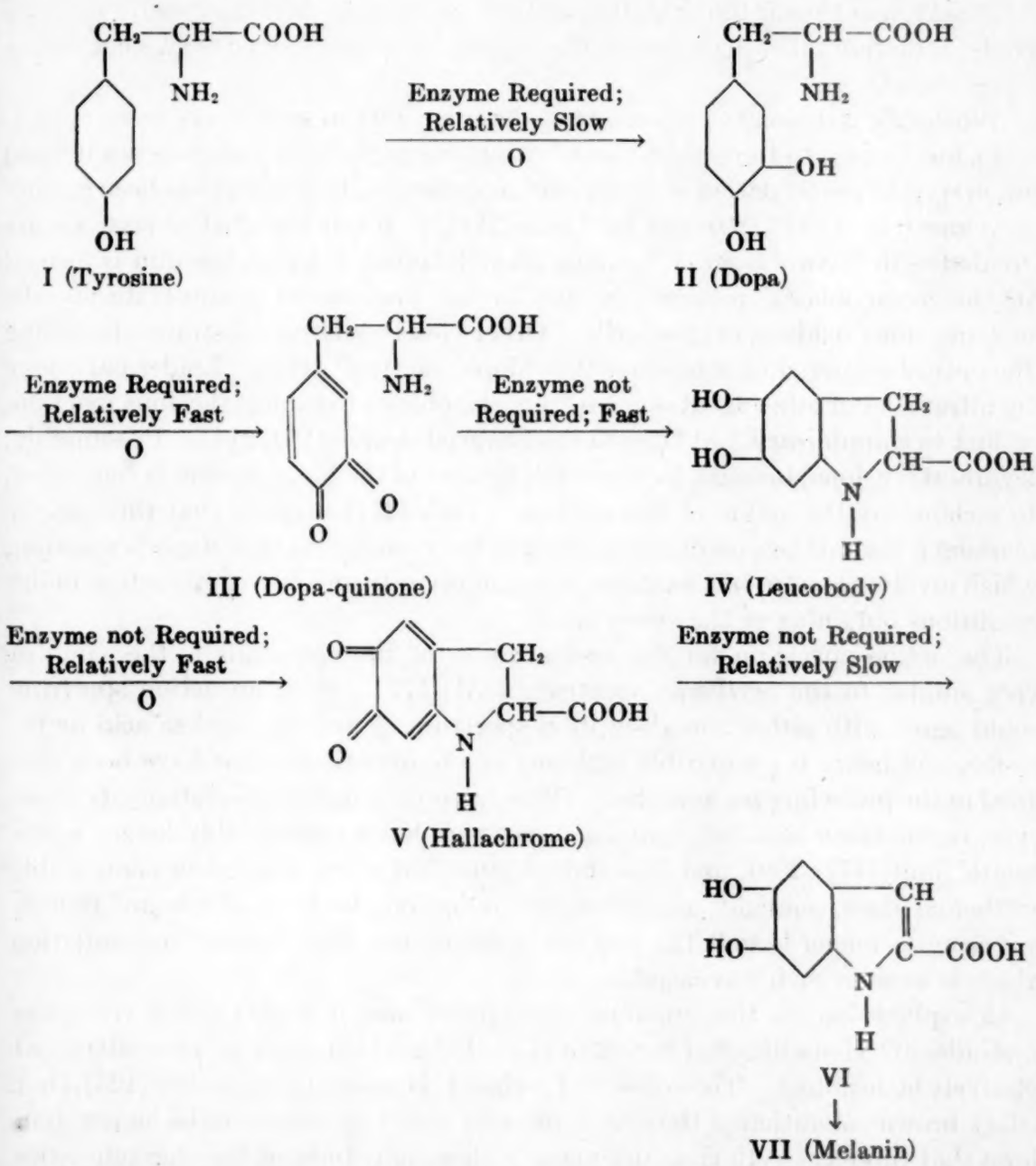

Fig. 4. Reaction scheme for the *in vitro* formation of melanin from tyrosine. (From Evans and Raper, 100.)

of suntan—a matter of days—would have to be occupied by the first reaction (I to II) in Raper's scheme, and it is doubtful that it would fill the time between exposure and the elaboration of melanin in the melanoblasts, which does not begin until well after suntan has made is appearance due to migration of the preformed pigment. The melanoblasts are exposed to relatively little

sunburn-producing ultraviolet radiation because of their deep position in the epidermis. The *in vitro* transformation of tyrosin to dopa proceeds with the uptake of molecular oxygen, whereas, in contrast, the subsequent appearance of suntan is not inhibited by cutting off the oxygen supply during the exposure (44, 131); and thus if the oxidation of tyrosine to dopa during exposure to ultraviolet radiation takes place *in vivo* the oxygen must be obtained from some source other than O_2.

Bloch (25, 26) assigned a somewhat different, and in most ways more acceptable, mechansim to the elaboration of cutaneous pigment, which does not depend upon specific participation of ultraviolet radiation. His concept has been further developed by Lutz (180) and by Peck (214). When histological sections are treated with levorotatory β-3-4-dioxyphenylalanine (l-dopa), melanin is formed in the melanoblasts, presumably due to the presence of a substrate specific enzyme, dopa-oxidase, in these cells. Other closely related substrates, including the optical isomer, do not produce this "dopa reaction" (215). Epidermal injury by ultraviolet or other agents causes the melanoblasts to exhibit the dopa reaction, at first to a diminished, but later to an enhanced degree (180, 214). Presumably, levorotatory dopa, brought to these cells by way of the blood stream is converted, to melanin by the action of this oxidase. Peck (214) suggests that this specific reaction is favored by conditions existing in the tissues, and that Raper's reaction, which involves less specific oxidases, does not occur to an appreciable extent under conditions obtaining in the epidermis.

The action spectrum for the melanization of the epidermis is the same or very similar to the erythema spectrum (131, 177). Such an action spectrum could agree with either the absorption spectrum of protein, nucleic acid or tyrosine, and hence is compatible with any of the hypotheses that have been outlined in the preceding paragraphs. There have been numerous statements, however, to the effect that the "tanning" spectrum has a considerably longer wavelength limit (177, 250), and it is indeed true that when applied in comparable erythemal dose, sunlight or carbon arc radiation, both of which are rich in wavelengths longer than 0.32μ, produce a deeper tan than mercury arc radiation which is weak in such wavelengths.

An explanation for this apparent discrepancy was provided a few years ago in studies by Henschke and Schultze (131, 132), which seem to have attracted relatively little notice. They observed, as had I. Hausser a year earlier (123), that a dark brown coloration of the skin is brought about by wavelengths longer than those that produce erythema, and made a thorough study of the characteristics of this phenomenon (131, 132). This response, which undoubtedly represents the darkening of preformed melanin and which will be referred to herein as *pigment darkening*, differs in several respects from the primary melanization that follows sunburn: The action spectrum for pigment darkening extends from about 0.30μ to 0.42μ, with a broad maximum near 0.34μ (curve *PD*, fig. 3); thus, in contradistinction to erythema and pigment formation, it is readily brought about by sunlight passing through window glass, which removes wavelengths shorter than 0.32μ. Pigment darkening may appear within the first few minutes

of exposure to sunlight, and usually reaches its maximum within an hour, whereas melanization is not manifest until a few days later. Several-hundred-fold greater dosage of radiant energy is required to bring about pigment darkening than is required to cause erythema and melanization. Pigment darkening is most pronounced in skin that has been previously sunburned and still retains traces of suntan, whereas melanization is most pronounced in skin not previously sunburned. Pigment darkening does not occur if O_2 is removed by blanching the skin by pressure with a quartz plate, whereas erythema and melanization are not affected by this treatment. Histological examination shows that these longer wavelengths do not cause pigment migration, nor the formation of new melanin (115).

It is probable that the tanning spectrum described by Luckiesh and Taylor (177) represents a combination of the action spectrum of this pigment darkening process with that of melanization, since those investigators did not differentiate the two processes.

Miescher and Minder (200), who confirmed and extended the findings of Henschke and Schultze, point out that either ultraviolet radiation (171, 188) or heat (170, 171) brings about darkening of pigmented cadaver skin, and that this seems entirely comparable to the pigment darkening in the living tissue. Miescher and Minder, who showed that all three phenomena take place only in the presence of O_2, believe that a common mechanism is involved; this they suggest is the oxidation of pigment already present in the skin in a reduced leuco-form. It is possible that the "melanoid" pigment described in human skin by Edwards and Duntley (84) as a result of studies of spectral reflection, is a leuco-form of melanin, or a mixture of the oxidized and reduced forms. Pigment darkening has been indicated in the scheme in figure 2, as a photochemical reaction directly involving the melanin in the epidermis; the leuco-form of melanin might well be the light absorber for this process.

Henschke and Schultze (132) showed that a relatively large proportion of the suntan produced by sunlight may be due to pigment darkening, because of the great proportion of wavelengths between 0.30μ and 0.42μ as compared to those shorter than 0.32μ (see figs. 1 and 3). This should be particularly true when the sun is far from zenith as in late afternoon or early morning, and at noon in late fall or early spring in temperate latitudes. It is possible that the pigment darkening effect accounts for the fact that many individuals retain a relatively dark suntan throughout the winter months, and the absence of this process may be an important factor in the pallor that develops in those seldom exposed to sunlight, a condition too frequently taken to indicate anemia.

Darkening of previously sunburned areas of the skin can be brought about by the administration of sex hormones (86, 112, 113), or other agents (146). The mechanism underlying this change in color has not been elucidated; it may represent either melanization or pigment darkening. The phenomenon is of particular interest because it illustrates the complexity of suntan, and of the factors that determine skin color.

Acquired immunity to sunburn. It is commonly recognized that the skin

becomes more resistant to sunburn after an initial exposure to sunlight. Some time just prior to 1900 (101), Finsen performed an experiment to determine the cause of this acquired immunity, his interpretation of which was so seemingly conclusive that it was at once accepted. As a result, a false concept was engendered which still persists. Finsen covered a part of the arm with India ink, leaving the remainder uncovered, and exposed the whole arm to sunlight for 3 hours. The uncovered part was sunburned, but the covered part remained unchanged. After several days, when the sunburned area of the arm had become tanned, he again exposed the arm to sunlight without any covering. After this second exposure, that area developed sunburn which had been protected by the ink during the first exposure, whereas the previously sunburned areas were scarcely affected. Finsen attributed the acquired immunity to screening by pigment developed as a result of the first exposure, which he thought mitigated the effects of the sun's rays by absorbing them. The idea that suntan directly protects against sunburn was generally accepted, and is widely held today.

About 1920, however, investigators began to point out that immunity to sunburn does not closely parallel the amount of visible suntan (152, 195, 217, 235, 236, 258), and it is now clear that melanin can play only a secondary rôle in acquired immunity to sunburn. Particularly convincing proof that such immunity may be acquired without any pigmentation whatsoever comes from two sources. The non-pigmented areas of the skin of vitiligo patients become more resistant to ultraviolet radiation as a result of exposure to that agent (195, 258), without any melanization occurring in these areas; and albinos, who do not form melanin, also become less sensitive after exposure (174, 236).

Observations by Guillaume in 1926 (111) indicated that relative immunity to sunburn is conferred by thickening of the horny layer of the epidermis. This finding was supported by Lovisati in 1929 (174), and in 1930 Miescher (196) carried out a thorough histological study, showing that marked thickening of the corneum occurs as a result of the epidermal hyperplasia brought about by the action of ultraviolet radiation. Measurements of the transmission of the ultraviolet radiation by the corneum, since obtained (see p. 487), show that this layer is very opaque to the wavelengths that cause sunburn, so that small increases in thickness should provide markedly increased protection for the living epidermal cells. The extreme resistance of the palms of the hands and the soles of the feet, which have little pigment, is no doubt due to the very thick layer of corneum they possess. Schall and Alius (235, 236) found that the maximum reduction of sensitivity of sunburned skin appears about one week after the exposure, and that normal sensitivity is regained in about 50 to 60 days. Hyperplastic thickening of the epidermis might reach its maximum in the former period, and normal thickness be regained by the end of the latter, suggesting that thickening of the corneum is the major factor in conferring immunity. Suntan, on the other hand, may persist for many months after the immunity is lost.

While all this evidence indicates that thickening of the corneum is the major factor determining the relative immunity to sunburn following exposure, the possibility that melanization and other factors play a rôle cannot be categorically

denied. The amount of radiation transmitted by the corneum is a function not only of the thickness, but of the attenuation coefficient, which in turn is a function of both absorption and scattering (see p. 487). Thus, the transmission of the corneum might be altered in three different ways: (a) by changes in the absorbing components, e.g., the accumulation of melanin; (b) by thickening, which results from epidermal hyperplasia; and (c) by alteration of the scattering charateristics. As pointed out above, the first of these factors is no doubt the most important; the relative importance of the second and third is more difficult to assess. Melanin is deposited largely in the basal-cells of the epidermis of the white races, although some of it migrates into the more superficial layers (see p. 498). Melanin absorbs the sunburn radiation strongly (27), and should afford a certain degree of protection whether in the corneum or in the malpighian layer itself, where it may act as an "internal filter". In the skins of negroes, the pigment is much more uniformly distributed throughout the epidermis than in the skins of the white races, and this, no doubt, accounts in part for the relative immunity of the negro to sunburn (198), although it is also possible that the corneum is thicker in the negro race than in the white. Migration of melanin into the more superficial layers may be an important factor in conferring relative immunity to sunburn during the first few days following an exposure, before the corneum becomes appreciably thickened. Observations by Hausmann and Spiegel-Adolph (127) suggest that alteration of the proteins of the corneum by ultraviolet radiation may decrease the scattering, and hence the transmission by that layer. It is conceivable that the transmission of the corneum might be influenced by its moisture content, since the amount of water between the elements of which it is composed may affect the scattering function. Measurements by the writer have shown, however, no appreciable effect on the erythema threshold to result from the application of water to the skin's surface, nor from sweating (43). The latter finding is somewhat surprising, since components of both sweat and sebum absorb the sunburn-producing radiation to some extent (68, 197).

Development of immunity to ultraviolet radiation by the epidermal cells themselves was first suggested by Perthes (217). Strong evidence to the contrary is to be found, however, in experiments by Miescher on the cornea of the rabbit eye. When exposed to ultraviolet radiation at regular intervals over a 28 week period, the sensitivity of the cornea to that agent did not change (197). The cornea does not possess a layer comparable to the corneum, to thicken and thus protect the cells of the corneal epithelium. Perthes (217) based his belief in cellular immunity on the apparent rapidity with which sensitivity to sunburn decreased after exposure. He found that a second dose of ultraviolet radiation produced less effect when it fell on a spot already exposed to that agent, even so short a time as one hour previously. Schall and Alius (235) showed, however, that incomplete summation of fractionated doses may lead to erroneous conclusions in such experiments, and may account for Perthes' finding and numerous other conflicting statements regarding immunity to sunburn that are to be found in the literature. Schall and Alius believe, however, that decreased sensitivity

first appears as early as twelve hours after exposure—too early to be accounted for by either migration of melanin or hyperplasia. Development of immunity at such an early period after exposure is difficult to explain.

Many ointments and lotions have been devised to provide artificial protection against sunburn, but these vary widely in their effectiveness. Any material capable of absorbing radiation between 0.29μ and 0.32μ should afford such protection, but there are numerous other additonal factors, e.g., the degree of scattering, which determine the effectiveness. There is variation in the protection afforded to different individuals by a given preparation, and this is related to the individual erythemal threshold (43). Window glass and some plastics afford good protection against sunburn.

Quantitative aspects. The degree of erythema developed in response to a given dose of ultraviolet radiation is a function of a number of variables, many of which have been discussed in preceding pages. As a result, quantitative measurements and comparisons are subject to considerable uncertainty. The importance of the transmission of the corneum in determining the amount of radiation reaching the living cells has already been discussed at length; the sensitivity of the living epidermal cells themselves to ultraviolet radiation, and the sensitivity of the minute cutaneous vessels to the dilator substance, are factors that might be expected to vary as well (43). The course of development of the erythema varies from individual to individual (233), and this renders the erythemal threshold—measured at a given interval after exposure, according to the usual procedure—an arbitrary index at best. When the complexity of sunburn is considered, it is not surprising that many conditions, external and internal, affect it. A number of these have been discussed in preceding pages, and a few more may be mentioned.

There have been numerous studies to determine the effect which heating the skin may have on sunburn, the results of which often appear contradictory. Investigations by Schall and Alius (234) and by Clark (57) indicate that during the exposure an increase in temperature has little effect on the erythemal threshold, as might be expected for a photochemical reaction, but shortens the time to appearance of the erythema. Confusion in previous studies may have resulted from failure to separate these two aspects of the response.

Sulfanilamide, when injected into the skin in high concentrations, lowers the erythema threshold (30, 99) probably by altering the susceptibility of epidermal cells to the radiation. Morphine injected intradermally produces the opposite effect, but in this case by absorbing the sunburn producing radiation (227). No doubt other drugs affect the erythema threshold. Sensory (4, 226) or sympathetic (203) denervation alters the response to sunburn-producing radiation, probably due to changes in vascular response.

The erythemal threshold varies considerably among individuals, in the same individual from time to time, and from one skin area to another, adding to the difficulty of comparative study. It is common belief that there is a close relationship between complexion and susceptibility to sunburn, red blonds being considered most susceptible, dark brunets least, but although there is certainly

a general relationship of this kind between complexion and the erythema threshold (97), anyone who makes measurements of a large number of individuals must be struck by the frequent exceptions to any rule. Certainly the thresholds of negroes of pure lineage are much higher than those of whites (124, 198).

Ellinger (97) finds characteristic seasonal changes, the threshold falling during the summer; he also attempts to correlate the erythemal threshold with the physical state of the individual, particularly with the activity of the thyroid gland.

The erythemal threshold is not necessarily an accurate measure of the amount of discomfort an individual may experience as the result of a given dose of sunburn-producing radiation, since there may be marked qualitative differences in the response of individuals. Even among those who respond so abnormally to sunburn that they are diagnosed as photosensitive, the threshold is often within the normal range (29, 126).

Quantitative and qualitative differences in individual response account for only a part of the apparent vagaries of sunburn as they appear to the casual observer, who is often unaware of the great variations in intensity of the sunburn-producing wavelengths of sunlight. Those wavelengths shorter than 0.32μ constitute less than 0.2 per cent of total sunlight (see fig. 1), and a most variable part of it. This component is subject to much greater change with the amount of atmosphere through which the sun's rays pass than is the visible component, because of greater attenuation by the atmosphere (p. 485), and hence the former varies much more with time of day, latitude, and season. It is only natural to evaluate the intensity of sunlight in terms of light perceived by the eye, but this organ does not record the intensity of the sunburn-producing radiation—thus the latter may be overestimated or underestimated at one time as against another. Sky-radiation, i.e., scattered solar radiation from the sky, is another confusing factor. At noon on a very clear day in temperate latitudes, the sky-radiation constitutes only about 10 to 15 per cent of the visible component falling on a horizontal surface, whereas for wavelengths shorter than 0.32μ, the direct and sky-radiation are about equal (178, 218); obviously one need not be directly exposed to the sun to receive a sunburn if he is sufficiently exposed to the sky. On a lightly overcast day, particularly in a fog, the scattered sunburn-producing radiation may be many times the direct; and one may, to his surprise, be sunburned under such conditions. At high latitudes at midday, the sunburn-producing component of the sky radiation is also much greater, relative to that of the radiation coming directly from the sun, than at low latitudes (60).

Another factor not generally recognized is that slight amounts of smoke or dust that are not particularly noticeable to the eye, may wipe out the wavelengths shorter than 0.32μ, and smoke polluted fog may provide very good protection against sunburn. Reflection from snow or ice may be an important factor, as is testified by the terms "snow-burn" and "glacier-burn"; reflection from water seems to be less important (64). These variables probably account for many false impressions regarding sunburn.

Cancer. The evidence that sunlight is a major factor in, or even the pre-

dominant cause of human cutaneous cancer, has been considerably strengthened by the results of recent experimental and epidemiological studies. The concept itself is not new, having been introduced at about the turn of the century. Earlier studies consisted entirely of attempts to correlate clinical findings with extent of exposure and susceptibility to sunlight; but subsequent to 1928, when Findlay first showed that cutaneous tumors of mice and rats could be induced by exposure to mercury arc radiation, experiments have been numerous. At present, five major lines of evidence support the concept: (a) cancer of the skin occurs principally on the parts of the body most exposed to sunlight (about 95 per cent on the face and hands); (b) cancer of the skin is more common among outdoor than among indoor workers; (c) the incidence of cancer of the skin is greater in regions of the earth that receive high insolation; (d) cutaneous cancer is less prevalent in negroes than in the white races, presumably because the former are less susceptible to sunlight; and (e) cancer of the skin of laboratory animals (mice and rats) can be induced by exposure to ultraviolet radiation. The writer reviewed the then available evidence on these points in 1940 (28), and will confine the present discussion largely to more recent findings.

Close relationship between the sunburn and carcinogenic mechanisms is suggested by the fact that both have the same long wavelength limit at about 0.32μ (28, 104, 223, 232). Hence, it seems reasonable to relate the carcinogenic mechanisms to the same fundamental injurious action on cells that is associated with sunburn, and is characteristic of ultraviolet radiation of wavelengths shorter than this limit. The attractive alternative hypothesis has been offered, that the ultraviolet radiation brings about somatic mutations by direct action on the nucleic acid of the chromosomes (181). Supporting this hypothesis is the fact that ultraviolet radiation is very effective in inducing mutations in unicellular organisms, (140) and in sex cells of Drosophila (71, 181), the action spectra of the former indicating that nucleic acid is the light absorber. The difficulties encountered in determining the true action spectrum for tumor induction are, however, even greater in this case than in that of sunburn, and it is not likely that a choice can be made between the two hypotheses on the basis of such measurements (32). It has been suggested that ultraviolet radiation acts on the steroids of the skin to produce a chemical carcinogen, but recent evidence indicates that this is very unlikely (22, 38). The chronic irritation hypothesis of cancer induction—less popular today than in the past—receives some support from the chronic "precancerous" changes that are associated with human cutaneous cancer. However, although chronic changes also occur in mice that are exposed to ultraviolet radiation, these changes are not necessarily preliminary to tumor formation since there is evidence that tumor cells are actually present very early in the course of the exposures, possibly immediately after the first exposure (34, 37). Other chronic lesions of skin have also been attributed to continual exposure to sunlight (126).

In writing the above paragraph the findings on the mouse were applied to man without hesitation. It is implicit in this application that the long wavelength limit for carcinogenesis would be the same for man as that found ex-

perimentally for mice and rats, a likely relationship, since the same long wavelength limit is characteristic of a large group of injurious biological effects (p. 491). The validity of the assumption is a matter of some importance, particularly in considering the geographical distribution of human cutaneous cancer, and hence an apparent discrepancy and its cause need mention. Malignant tumors of human skin are virtually all epithelial, i.e., carcinomas, but in apparent contrast to this, sarcomas—non-epithelial tumors—predominate among the tumors induced in mice by ultraviolet radiation (107). This apparent lack of agreement between the case of man and that of the mouse is explainable in terms of differences in penetration of the ultraviolet radiation into the skin of the two species (155). Those wavelengths shorter than 0.32μ are virtually all absorbed in the epidermis of man, and it is here, where only epithelial cells are present, that human cutaneous cancer appears. In the mouse, on the other hand, this radiation penetrates much deeper, to reach connective and other non-epithelial tissues, accounting for the high proportion of sarcomas found among the tumors induced by ultraviolet radiation in these animals.

Until recently, data on incidence of human cutaneous cancer with respect to geographical latitude have not been too convincing, but recent studies by Dorn (77) indicate a clear north-south distribution. This investigator has made a careful analysis of the incidence of various types of cancer occurring in certain regions of the United States, which he groups as North, West, and South, but which actually form a series with respect to latitude, the weighted averages being 41°, 38°, and 32° for north, west and south respectively. Dorn's findings are particularly striking in that cancer of the skin and cancer of the buccal cavity (largely cancer of the lip), show a clear-cut north and south distribution. For example, the incidence of cutaneous cancer in the white male populations was 23.1, 48.5, and 116.4 per 100,000 for north (41°), west (38°), and south (32°), respectively. Similar distributions occur for white females and for cancer of the buccal cavity for both sexes. Such a north-south distribution was not found for cancer occurring at other sites in the body, and hence it appears that some factor related to latitude is involved in the occurrence of tumors of the exposed parts, the skin and the lip.

On first consideration the wide variation of cutaneous tumor incidence with latitude seems difficult to account for, because one is apt to think in terms of visible sunlight, which does not vary so greatly with latitude (see p. 485). Ultraviolet radiation shorter than 0.32μ varies much more widely with latitude, however, as reference to figure 1 and equation (1) will illustrate. The values of m_λ in equation (1) are much greater for the sunburn producing radiation than for that perceived by the eye, which means that the former will vary much more with the zenith angle of the sun, and hence with latitude and time of day, than visible radiation. The variation is not great enough, however, to account alone for the variation in incidence of cutaneous cancer with latitude. Experimental findings on mice help to explain this discrepancy; since (a) intensities of ultraviolet radiation below a certain critical value have relatively little effect (33), which would mean that the length of the day during which this agent can

be effectively carcinogenic is limited to the time during which the intensity is maintained above a critical limiting value; and (b) the time between exposures to ultraviolet radiation is a very important factor in tumor induction (35, 41), rest periods permitting partial or complete recovery (34, 37). With increasing latitude the number of days during the year on which the intensity of the radiation is above the critical value decreases, and thus there will be a longer rest period each year during the winter months which should lead to much slower development of cutaneous tumors. An exact quantitative analysis is not feasible, but all these factors would tend to increase the influence of latitude on cutaneous cancer incidence.

Dorn's data also indicate a lower incidence of skin cancer in negroes as compared to the white population (77).

None of the five arguments presented is completely convincing in itself, but as the evidence accumulates, it converges in a striking manner to support the conclusion that sunlight is the major cause of cancer of the skin. Lest this conclusion should appear too alarming to some reader, the writer hastens to point out that cancer of the skin affects only about two-tenths of one per cent of the population under the most severe condition represented in Dorn's data, and that as a whole, cutaneous cancers are among the least malignant of cancers, with the exception of certain highly malignant, but rare, types that are probably not caused by sunlight.

Vitamin D. The formation of vitamin D, and the resultant antirachitic action, is the one clear cut, beneficial effect known to be produced by sunlight impinging upon human skin. Its relative importance would, therefore, seem to merit a more prominent position and greater space in this review, but most aspects of the subject are so well known, and have been so adequately treated elsewhere that extensive discussion would be superfluous.

Vitamin D is formed by the action of ultraviolet radiation of wavelengths shorter than approximately 0.32μ upon 7-dehydrocholesterol or some very similar precursor steroid compound. The fact that this process has the same approximate long wave length limit as sunburn does not indicate any relationship between the two processes, and may be regarded, from a physiological point of view, as fortuitous. The action spectrum for antirachitic action has been determined for rats by Bunker and Harris (50) and by Knudson and Benford (156), and while the two sets of data differ to some extent, both indicate that the photochemical mechanism is definitely not the same as that of sunburn. Knudson and Benford's measurements of the action spectrum agree closely, and those of Bunker and Harris reasonably well, with the absorption spectrum of 7-dehydrocholesterol (36). This agreement suggests that the photochemical reaction takes place near the surface of the skin where there is little or no shielding by intervening layers as in the case of sunburn, and hence that the site of formation of vitamin D is probably in the corneum rather than in the living cells of the epidermis. That this is the site of formation is also indicated by the fact that a considerable amount of vitamin D is removed in bits of desquamating corneum which may be rubbed or washed off from irradiated skin (129).

Attempts to correlate the geographical incidence of rickets with the incidence of sunlight have not met with great success partly because adequate measurements of the antirachitic part of sunlight have not been available, but also because the picture is complicated by the dietary factor. In this respect it is of interest to note that in Greenland, rickets is unknown among the Esquimaux (23), who certainly receive little antirachitic radiation.

Photosensitization. Quantitative and qualitative variations in susceptibility to sunburn have already been mentioned. When these deviations from the norm are sufficiently great they may be considered as pathological conditions, and are properly designated as *polymorphic light eruption*, the term indicating the variability of the responses included in this category (29, 126). Other idiopathic types of sensitivity to sunlight occur in which the sunburn mechanism is not involved, but these are rare (29), and lie outside the scope of the present review. In addition there are forms of sensitivity to sunlight induced by photosensitizing materials, including certain drugs, the photosensitizer entering the body by contact in some cases, by ingestion or injection in others (29).

There is a particular type of photosensitization, given the name *photodynamic action* which is of interest, here, chiefly because it is sometimes invoked in explanation of experimental findings to which it does not apply. This type of photosensitization is produced by the introduction of a wide variety of dyes or natural pigments into living systems. Destructive processes are then brought about when the sensitized tissues are exposed to light, which may be manifold in their expression but which have a common mechanism: under the action of light, oxidation of readily oxidizable substances in the photosensitized system takes place with the uptake of oxygen; the effects usually are injurious. The role of the dye in this reaction is to capture light quanta and transfer the energy, so gained, to the reaction, the dye itself remaining chemically unchanged at the end (29).

The porphyrin pigments are among the active photosensitizers of the photodynamic type, and it has been thought that they play an important rôle in the physiological responses of the human body to light. There is no evidence for this, however, beyond the fact that massive doses of hematoporphyrin will, like numerous other photodynamic dyes, cause severe photosensitization if introduced into the blood stream. It is even doubtful that such relatively high concentrations of porphyrin as appear in the body in certain pathological conditions cause photosensitization, general belief to the contrary notwithstanding (29). Conclusive evidence against the idea that prophyrins have any rôle in the production of sunburn in normal individuals lies in the fact that the wavelengths to which they sensitize do not agree with those that cause sunburn (29), and that photosensitization of the skin by porphyrin is inhibited by the absence of O_2 as is characteristic of photodynamic action but not of sunburn (44).

Porphyrins derived from chlorophyll and certain other pigments found in particular species of plants are the cause of photosensitization in domestic animals, a matter of great economic importance in some regions of the earth, but there is little or no evidence that comparable conditions exist in man.

Photosensitization of the photodynamic type does occur in man in some instances due to drugs or industrial products (see 29).

There is an erroneous belief that photobiological effects produced by ultraviolet radiation may be brought about by longer wavelengths when a photosensitizer is present. For example, there have been a number of unsuccessful attempts to accomplish the cure of rickets by using a photodynamic dye and visible light (see 29, pp. 118–120).

Temperature effects. The stimulation of cutaneous sensations by radiant energy impinging on the skin has been the subject of recent extensive studies by Hardy and his co-workers. Three levels of sensation are elicited by successively greater intensities of radiant energy; warmth, heat and pain. Maximum sunlight is of sufficient intensity (slightly less than 0.03 gram calories cm^{-2} sec^{-1}) to elicit the sensation of warmth or heat, depending upon the part and extent of the body it strikes (119, 133, 134, 135), but never reaches the threshold of pain sensation (120). The amount of radiant energy required to elicit the sensation of pain must be exceeded by about ten times (120) before actual damage to the skin occurs, and hence the persistent erythema and pigmentation reported to occur from intense exposure to infrared radiation from other sources is not to be expected from the most intense sunlight.

The actual stimulus for cutaneous sensation produced by radiant energy appears to be the alteration of the temperature gradient in the superficial layers of the skin (133, 210). Effectiveness in accomplishing such a change in gradient varies with the wavelength of the radiation; visible and near infrared radiation (0.36μ to 3.0μ) being partially reflected, and hence less effective in terms of incident energy than far infrared ($>3.0\mu$)[5] which is entirely absorbed (209). Moreover, the far infrared, because it is more strongly absorbed, is more effective in altering the temperature gradient in the superficial layers, and hence is a better stimulus for cutaneous sensation.

A comparison between white and negro skin made by Hardy and Oppel (209) is particularly interesting in this respect. Both types of skin absorb the far infrared completely and very superficially, and to such radiation the skins of the negro subjects were somewhat less sensitive than the skins of the white subjects, apparently because of an intrinsically less sensitive cutaneous sensory mechanism on the part of the former. On the other hand, negro skin is a better absorber of sunlight than is white skin (see table 1), and the negro subjects were found to have a threshold to incident sunlight somewhat lower than that of the white subjects.

Sunburn causes a general hyperesthesia of the exposed area; the skin becomes more sensitive to touch and to temperature, and a mild stimulus such as contact with clothing often elicits the sensation of itching. Shumacher (240) found that the pain threshold was lowered 24 per cent, on the average, by a moderately severe sunburn.

Even far infrared radiation that virtually does not penetrate below the skin's

[5] These are the approximate spectral ranges of the radiation used by Oppel and Hardy (209).

surface can alter the temperature gradient in the skin to a considerable depth (163). Under ordinary room conditions the temperature of the skin surface is a few degrees below the interior of the body, the extent of the difference depending upon the temperature of the room, but when radiant energy having the intensity of sunlight impinges upon the skin surface this situation may be reversed. The gradient is greatly affected by the circulation, which tends to distribute the heat throughout the body. The importance of this factor is shown by occluding the circulation while the skin is exposed to radiation, the temperature of the surface layers then rising. The importance of the circulation through the peripheral blood vessels in determining the skin temperature is shown in the case of the erythema of sunburn—temperatures of the surface layers being raised considerably above those for non-erythematous areas in the same temperature environment (163). It has often been suggested that melanin pigment in the skin has an important effect upon the distribution of heat. Both the measurements of Keller (153) and of Laurens and Foster (163) show that pigment has no such effect on long wavelength infrared radiation, as might be expected from the fact that this radiation virtually does not penetrate below the skin surface. The visible and short infrared, of which sunlight is largely composed, are absorbed somewhat more superficially in heavily pigmented skins than in those meagerly pigmented (153, 163), but the influence on the temperature gradient would not seem to be great, as compared to that of the circulation.

Dilatation of the superficial vessels and sweating both provide channels of heat dissipation in hot environments, the latter being the most important in extreme conditions. The relative importance of the cutaneous sensory receptors in bringing about adjustments of these two functions, as compared to the central regulatory mechanisms stimulated by heating of the blood, is not altogether clear (see 19, 52). It may be pointed out that so far as the cutaneous sensation of warmth is concerned, sunlight is a considerably less effective stimulus than the radiation from a hot stove (209), and this together with the variation in the threshold in different areas of the body, would seem to render this sensory mechanism a rather inaccurate index of temperature relationships of the body as a whole. The mechanism of vasodilatation in the local areas exposed to heat, not pronounced as a rule in exposure to sunlight, has yet to be completely elucidated (e.g., see 103). Local heating of the skin is not very effective in producing local sweating, the control of this process being mediated chiefly through central mechanisms (160).

Other effects. As our knowledge of the few clearly established cutaneous responses to sunlight increase, there seems correspondingly less reason to credit the numerous claims for additional effects. Most evidence supports the view that wavelengths longer than 0.32μ have no specific photochemical effect on normal skin, other than the pigment darkening effect, which extends to about 0.42μ. There have been reports, however, that ultraviolet wavelengths longer than 0.32μ may bring about erythema (124, 199), and there is also some evidence that such wavelengths affect living systems in general. Lethal effects on

bacteria and other lower forms brought about by the longer wavelengths of the ultraviolet, have been the subject of recent studies by Hollaender and his coworkers (139, 148), whose findings indicate an essentially different character for these effects than for those produced by wavelengths shorter than 0.32μ. The writer (29, p. 117–118) has suggested that such processes may represent photodynamic action, for which point of view there is some evidence in earlier literature. The possibility that such effects are common to all living systems must be entertained, but it seems improbable that they will prove to constitute an important cutaneous response to sunlight.

It is possible that sunlight may destroy photolabile vitamins, e.g., vitamin A, and riboflavin, in the skin; but it seems improbable that this is important in the bodily economy, particularly in the clothed man.

Hill (136, 137) believed he had shown that long infrared radiation impinging on the skin caused swelling of the mucous membranes of the nose ("nose closing effect"), whereas red or short wavelength infrared caused shrinking of these membranes ("nose opening effect"); but the ample studies of Dufton and Bedford (80) and of Winslow, Greenburg and Herrington (257) have not confirmed these findings. Nose closing appears to result from heating of the skin by any means, including sunlight (80). Some individuals are apt to sneeze when they go from dark interiors to the sunlight, a reflex which may be associated with this phenomenon or possibly with visual stimuli, but which does not seem to have been adequately explained.

Walthard and Spörri (253) describe changes in the chronaxie of skin exposed to "red", "blue" and "ultraviolet" light. Their data suggest that the red and blue effects (no doubt considerable infrared was included in the latter) were probably associated with temperature changes in the skin; while the ultraviolet effects, which appear to be of a different character, were associated with sunburn.

Ehrenwald (87, 88, 89) found that exposure of the skin to radiation from incandescent lamps, either directly or through glass filters, brought about certain postural changes. This effect, if substantiated, may be of interest with regard to the participation of cutaneous sensory mechanisms in postural reflexes, but Ehrenwald's contention that it represents a specific cutaneous sensory mechanism dependent upon the wavelength of the light, is not justified. The spectral energies were not properly evaluated, and there seems no reason to assume that any sensory mechanism other than thermal was involved. As pointed out in the preceding section, the intensity of thermal stimulation is to a certain extent a function of wavelength because of differences in absorption.

Effect on the eyes. To examine the process of vision would be beyond the scope of this review. A brief discussion of injurious effects of sunlight on the eye is in order, however. A number of these have been claimed, some without factual support, the inadequacy of the evidence having been pointed out by Verhoeff and Bell (251). The paper which these authors presented in 1916 must be regarded as a classical work on the subject, although, to judge from the relative infrequency with which it is quoted, it does not seem to have received as wide recognition as it merits. In it are described critical experiments on most as-

pects of injury to the eye by sunlight, and by radiation from artificial sources. Only in minor details is reinterpretation in terms of more modern concepts necessary, and the general conclusions appear as acceptable today as when they were formulated.

Ultraviolet radiation of wavelengths that produce sunburn causes injury to the cells of the cornea and conjunctiva, which results in the condition known as photophthalmia. This condition is characterized by pain, visual disturbances and photophobia, with excessive secretion, edema, and purulent discharge, according to the severity of the injury. Although there has been no attempt to determine a complete action spectrum, the long wavelength limit has been set approximately by Verhoeff and Bell at about 0.305μ. By analogy with sunburn and other effects of ultraviolet radiation it may be judged that the primary action is on the protein, or possibly the nucleic acid of the cells. As in the case of sunburn of the skin there is a long latent period between the exposure to ultraviolet radiation and the first symptoms. From these and other resemblances, there seems every reason to regard photophthalmia as sunburn of the eye. Unlike sunburn of the skin, however, sunburn of the eye does not confer protection against subsequent exposure (197). This condition results frequently from exposure to artificial sources of radiation, particularly welding arcs, but may also occur as a result of prolonged exposure to intense sunlight. "Snowblindness" or "glacierblindness" is generally attributed to this cause (251), but it seems probable that subjective visual effects also play a rôle therein. It is not improbable that some of the severe visual symptoms described are associated with the photophobia which is a part of the symptom complex of photophthalmia.

Photophobia, i.e., pain accompanied by attempts to avoid light when there is a sudden increase of intensity, is an accompaniment of numerous pathological conditions of the eye. The retina is apparently the receptor of the stimulus, since photophobia does not occur in blind eyes, and since the stimulating wavelengths are those that affect the retina (244). Siegwart (244) found that blind eyes did not manifest photophobia, but in cases of unilateral blindness where the consensual iris reflex was preserved, illumination of the normal eye resulted in pain associated with the blind eye. Siegwart (244) and Lebensohn (165) present evidence that the pain is associated with reflex movement of the iris muscles, and reflex dilatation of the vessels of the conjunctiva. The reflex changes which lead to the pain involve the trigeminal nerve, and are abolished by removal of the Gasserian ganglion. It would seem, thus, that photophobia is a normal response to too intense light, which is aggravated by pathological conditions of the conjunctiva.

A fairly high incidence of tumors of the eye occurs in mice and rats exposed to ultraviolet radiation (172, 223). Reasoning from the parallel case of cancer of the skin, one may suspect that sunlight plays a rôle in the induction of tumors of the superficial structures of the human eye (13, 161).

If a man looks directly into the sun for too long a time he develops a scotoma which may remain permanently; this phenomenon is generally called "eclipse blindness". The damage is undoubtedly due to heating of the retina at the point

at which the sun's image is focused, to a temperature that causes local tissue damage. About half the energy of sunlight reaching the retina is perceived by the eye, and about half lies in the infrared, the ultraviolet portion being negligible (40, 83, 121, 224). Hence the infrared, although it has no specific effect, cannot be disregarded in designing glasses for looking into the sun. In fact, glasses which cut off most of the visible radiation without comparable reduction of the infrared, as, for example, most deep red glasses, may be dangerous because they lead to a false sense of security. With the visual mechanism protected by a reduction of the intensity of those wavelengths that specifically stimulate it, the individual may look into the sun without discomfort, yet receive enough infrared radiation to burn the retina (83).

It is sometimes assumed that eclipse blindness should have higher incidence in tropical regions (98), but there is little reason for this to occur. The intensity of sunlight reaching the retina is only about 20 per cent less with the sun at 60° from zenith, than with the sun at zenith (40). Thus, so long as the sun is reasonably high in the heavens it is dangerous to look directly into it, no matter what the latitude, and it would seem necessary to account in some other way for any differences in the incidence of eclipse blindness in different geographical regions.

It is highly improbable that ultraviolet shorter than 0.32μ reaches the retina in sufficient quantity to have deleterious effect, since it is absorbed so strongly by the proteins of the ocular media. Some changes in the retina have been reported after intensive exposure to mercury arc radiation (81), but such dosage would not be received in exposure to sunlight. There have been numerous claims that the longer wavelength ultraviolet has harmful effects, but this has not been substantiated (243).

The high incidence of cataract among glass blowers is usually thought to be caused by the intense radiation from the glass blower's furnace. The mechanism by which the cataract is produced has been debated, however; heat, and specific effects of both ultraviolet and infrared radiation having been invoked. The belief that ultraviolet radiation is the exciting agent has been held by various workers (e.g., 51, 56) since Schantz and Stockhausen (237) first claimed that the radiation from the glass blower's furnace includes such radiation. Quantitative measurements of the spectrum of such a source by Vogt (252), indicate that it contains little or no ultraviolet radiation as is to be expected from its relatively low temperature (251). Hence, so far as glass blower's cataract is concerned, it seems unprofitable to debate the manner in which ultraviolet radiation may affect the lens. As regards the action of infrared, in which this source is rich, it seems that any effect produced on the lens is probably due to heating of that organ; but that the iris, rather than the lens, is the principal absorber of the radiation that causes the heating (16, 17, 121).

It has been reasoned, in view of the accepted etiology of glass blower's cataract, that sunlight may be a causal factor in senile cataract. Actually the two cases are far from parallel because of the great differences between the spectral distribution of sunlight and that of the radiation from the glass blower's furnace and the fact that the lens and iris are subject principally to diffuse rather than direct sunlight.

In contrast to the radiation from the glass blower's furnace, sunlight contains ultraviolet radiation of wavelengths shorter than 0.32μ. Such radiation, if intense enough, can produce injury to the lens epithelium and this might lead to cataract formation; and there is also the possibility that ultraviolet radiation may affect the protein of the lens directly as suggested by Clark (56). The intensity of radiation of these wavelengths in sunlight is not high, however, and the amount that penetrates through the cornea and aqueous humor to reach the lens must be quite small.

Differences in the primary site of development of senile cataract as compared to glass blower's cataract also suggest a different etiology (251). While it is conceivable that sunlight may play a rôle in the development of cataract in some cases, since to quote Bourne (46)—"no doubt there are as many ways of producing cataract as there are to injure the lens"—it would seem unlikely that it is an important factor in most. The high incidence of senile cataract said to occur in certain countries near the equator is probably to be explained in terms other than high insolation (98).

SYSTEMIC EFFECTS. It is difficult to estimate just which of the many systemic changes reported to be brought about by sunlight are specifically caused by the radiation, are of major importance, or are spurious. This uncertainty is due in many instances to lack of information regarding the intensity, dosage and spectral distribution of the radiation employed, and even when these factors are reasonably well described it is often difficult to assign a specific cause to a given effect. Because the interpretation of many of the reported observations is uncertain an exhaustive review of the literature will not be attempted, but only those points will be treated which are satisfactorily explained, or which have aroused particular interest.

The solar heat load. Of the total solar radiant energy striking the body, a certain fraction is reflected by the skin or by the clothing, the remainder being absorbed. The absorbed portion, which is here called the *solar heat load*, is composed of direct radiation from the sun, and reflected radiation from the sky and from the terrain. The relative and absolute values of these three factors vary widely according to the position of the sun in the heavens and with the position of the man with respect to the sun, as well as with the reflecting properties of the skin, clothing and terrain. Table 2 gives some estimated values for the solar heat load and its component parts under a limited number of conditions. Since certain assumptions have had to be made regarding the geometry of the man, and the reflection from the terrain, skin and clothing, the estimates are only roughly approximate (39). Reflection from the body has been assumed to be 43 per cent, a value found by Martin both for very blond skin, and for khaki cloth (183).

The relative magnitude of the solar heat load is perhaps best understood by comparing it with the metabolic heat load. The average of the values for the total solar heat load estimated in table 2 is about 4.0 kilocalories per minute or 240 kilocalories per hour. Thus the maximum solar heat load may be two or three times the resting metabolic heat load (about 96 kilocalories per hour) or about equal to the metabolic heat load in ordinary walking (about 265 kilo-

calories per hour). Obviously this factor cannot be disregarded in considering the total heat load.

The physiological importance of the solar heat load cannot be separated from the total heat load, of which it is a variable part. The total heat load for a body out of doors is the algebraic sum of heat gains and losses through a number

TABLE 2

*Estimated solar heat load under various conditions**

POSITION OF MAN	ZENITH ANGLE	SOLAR HEAT LOAD† KILO CALORIES PER MIN.			
		Direct	Sky	Terrain‡	Total
Erect	0°	0.90	1.13	1.84	3.87
	60°	2.67	0.45	0.74	3.86
Prone	0°	3.84	1.13	0.76	5.73
	60°	1.54	0.45	0.30	2.29

* From Blum (39)

† Under the following atmospheric conditions, 20 mm. H_2O, 2.8 mm. ozone, 300 dust particles per cm.^{3}, and the assumption that 43 per cent of the total solar radiation is reflected by the body and clothing.

‡ Albedo of terrain assumed to be 0.25.

TABLE 3

Attempted thermodynamic balance sheet for a man marching at 3 miles per hour; ambient air dry, with temperature about 37° C., terrain at 60° C., and body surface at 37° C.

	kilocalories per hour
Metabolism	+265
Total solar heat load	+234*
"Black body" radiation exchange with terrain	+128*
"Black body" radiation exchange with heavens	−128†
Evaporation	−506‡
Convection and conduction	± ?§
Total	−7 ± ?

* From Blum (39).

† Estimated by Blum (39) from calculations by Simpson (245).

‡ Based on an average value of 882 grams of water loss per hour for men marching under hot desert conditions (5). This factor is the one that must vary to maintain a balance if such is achieved.

§ These factors should be relatively small under such conditions.

of channels which are indicated in table 3. The values assigned in this table are very rough estimates for a set of conditions which would impose a relatively high degree of heat stress. The derivation of these values cannot be discussed here, and the reader is warned that they are inexact and that the close balance indicated is entirely fortuitous. The table was formulated and is presented here only to show that a trustworthy estimate of the relative importance of the solar

heat load can be made only in terms of a number of other factors, e.g., "black body" radiation to the heavens, which are themselves subject to variation (39).

The systemic changes imposed by the solar heat load as such, are the same as those imposed by heat load from any other source; they involve, primarily, a dilatation of the cutaneous vessels, and the stimulation of sweating. If the load is sufficiently great the effectiveness of these mechanisms in dissipating the heat load may be inadequate and the body temperature then rises. If water supply is limited, severe dehydration may occur due to sweating and this may lead to circulatory collapse, due chiefly to decreased blood volume (5, 6).

Circulatory effects. As has just been said, circulatory readjustments may be brought about as the result of increased heat load. There is also evidence that systemic circulatory changes result from sunburn. Laurens and his co-workers have carefully examined this subject in a series of studies which were summarized, up to 1933, in a monograph by that investigator (162). In these studies, high dosages of carbon arc radiation have been employed as a rule. In the dog, lowering of systolic and diastolic blood pressure follows exposure to dosages sufficient to produce persistent erythema; cardiac output is also decreased. In normal man the lowering of blood pressure is less in extent, and if anything, there is a slight increase in cardiac output (147). The fall in blood pressure cannot be explained by changes in blood volume, and according to Laurens' view, is the result of general peripheral arteriolar dilatation, due to the release of a dilator substance into the blood stream; the local release of dilator substance in sunburn has been discussed previously. The presence of such a histamine-like dilator substance in the blood of dogs after exposure to carbon arc radiation, has been correlated with the falling blood pressure (157, 164). Graham (108) has found that blood pressure lowering following carbon arc exposure is accentuated in adrenalectomized dogs, and that injection of desoxycorticosterone acetate or adrenal cortical extract abolishes the blood pressure lowering effect. Since the effect of histamine is enhanced by removal of the adrenal cortex and antagonized by adrenal cortical substances, Graham's findings may be interpreted to indicate that blood pressure lowering is due to the elaboration of histamine following carbon arc radiation. On the other hand, Graham also finds (109) that capillary permeability, as demonstrated by passage of dye from the blood into the tissues, is increased following exposure to carbon arc radiation, both locally and in the body generally, and that adrenal cortical substances inhibit this effect. Menkin (191) has shown, similarly, that injections of inflammatory exudates, or of leucotaxine, produce general capillary dilatation, and that this is likewise inhibited by injections of adrenal cortex. These later observations suggest that leucotaxine is released into the circulating blood from the sunburned skin (see p. 495), and that a generalized lowering of capillary permeability results, which may play a part in the blood pressure lowering effect. Weiss, Robb and Ellis (254) found that histamine does not produce in man the effect on blood pressure that characterizes its action in numerous other species, and that it is destroyed rapidly so that its concentration in the blood does not

rise to high levels (see also 225). Again we are faced with difficulty in attempting to identify the dilator substance associated with sunburn.

It is possible that other systemic effects result from the release of other inflammation-substances, and it seems unlikely that the total picture is greatly different from that produced by very superficial burns resulting from other causes.

Carbon arc radiation, which has been employed in most of these studies, contains wavelengths other than those that cause sunburn, being particularly rich in longer ultraviolet wavelengths in the region of 0.39μ where the characteristic cyanogen band emission occurs. The possibility of some specific effect of these wavelengths, which are also present in sunlight, must be entertained; but for the present there is no direct evidence that they play a part in the circulatory changes. The total intensity of the radiation used in some of these studies has been approximately equal to that of maximum sunlight, and hence the effects of increased heat load are not entirely excluded. These points need mention because some experiments have indicated that mercury arc radiation—which is poor in the long wavelength ultraviolet radiation and in total energy relative to sunburn producing wavelengths—is not as effective in eliciting such changes as is carbon arc radiation, which more nearly resembles sunlight (see 162).

As regards the formed elements of the blood, exposure to ultraviolet radiation seems to produce a slight increase in red cell, white cell and platelet counts, but there is disagreement among various reports, due probably to the lack of uniformity in the dosages and procedures employed (158, 162, 166). Release of Menkins' leucocytosis factor (190) from the inflamed skin might bring about an increase in white cells, and other changes in the formed elements may result indirectly from the inflammatory process.

Metabolism and growth. The measurement of metabolism after sunburn, when the cutaneous vessels of a considerable surface area are dilated, may be subject to error and misinterpretation (79), and there have been conflicting reports in this regard (162). Recently, Lippman (173) has reported a slight increase in basal metabolism, as measured by indirect calorimentry as had Lehmann (166) somewhat earlier. Lehmann (166) also described an increase in metabolism and in capacity for muscular exertion to accompany repeated exposures, but this seems to require confirmation. Release of fever producing substances from the site of inflammation in the skin may occur (193, 194). The production of vitamin D in the skin by ultraviolet radiation affects calcium and phosporous metabolism, but beyond this there seems to be no clearly demonstrated specific influence of sunlight on metabolism (162).

No specific effect of sunlight on growth of human beings is known beyond the influence that vitamin D may have. Many experiments purporting to demonstrate effects of light on growth of laboratory animals have been described, but it is probable that many of these represent changes caused by abnormal conditions of temperature rather than specific effects of light. When small animals are placed in closed cages covered with glass and exposed to sunlight,

as has been done in many experiments of this kind, the temperature of the interior rises, due to what is commonly called the "greenhouse" effect. The greater part of sunlight is transmitted by the glass, and this being absorbed by the walls of the cage is transformed into long wavelength "black body" radiation which cannot pass out through the glass. The result is that the temperature rises in the cage, and the animal is subjected to a large heat load by radiation from the walls of the cage to which must be added the heat load from the ambient air and from the direct rays of the sun. Forced ventilation of the cages does not ensure uniform temperature under such conditions, and may only add to the complexity of the situation. Rodents, the animals commonly used for such experiments, have poor ability to cool themselves by evaporation of water, and also have low total heat capacity because of their small size. Thus they are poorly equipped to combat high temperatures, which may, therefore, affect their metabolism and growth. If different colored glasses are placed over the cages, as has been the practice in experiments of this kind, different temperature conditions must obtain inside the cages because of the different amounts of the sun's radiation absorbed by the glass. The conclusion that under such conditions one has demonstrated a specific effect of, say, red light, because animals have grown at a different rate or behaved differently in other respects under a piece of red glass than under glass of some other color, is not warranted. Effects on plants under similar conditions have obviously no relationship to those on animals, because light plays an entirely different rôle in the economy of the two kinds of organisms, yet there has been an unfortunate tendency to make this inference.

Recent experiments have shown that the suppression of growth of mice by repeated heavy doses of ultraviolet radiation is due to decrease of food intake (42), and it seems probable that this results indirectly from irritation of the animal's eyes (167). There seems to be no real evidence that sunlight has any direct effect on the growth of human beings enjoying adequate diets.

Effects on endocrine systems. The sexual cycle of many kinds of birds and of certain mammals is influenced by light. It seems most probable that the eye is the receptor for the stimulus that brings about these changes, and that the pituitary is involved in mediating the effect (24, 59, 182). The principal effect of the illumination may be to prevent sleep, and to increase the activity of the animals (231). Certain experiments which appear to indicate that stimulating mechanisms other than the eye are involved (21) are open to objection as regards the methods used to measure and control the radiation. Recently, a hormonal mechanism has been proposed (47). It is occasionally suggested that a similar effect may occur in man (e.g., 168), and in support it is sometimes stated that the menses are suppressed in Esquimaux women during the months of the complete arctic night. This statement seems to come from observations by Cook (66), made during the first Peary North Greenland Expedition, but I have found no mention of such an effect by others who have observed the Esquimaux (e.g., 23, 221).

Ellinger interpreted certain of his experiments (95, 96) on mice to indicate

that excessive exposure to ultraviolet radiation brings about alteration of thyroid gland function, but no histological evidence of this has been found (42, 185).

Myerson and Neustadt (204) have reported that mercury arc radiation applied to the skin causes increased androgen excretion.

Therapeutic action. Curative effects of sunlight have been repeatedly described. These are usually attributed to the ultraviolet component, presumably those wavelengths shorter than 0.32μ, and it is generally assumed that the same effects are brought about by radiation from other sources rich in these wavelengths. Avitaminosis D can be cured by summer sunlight, or by radiation from other sources rich in wavelengths shorter than 0.32μ, but beyond its effect on this particular avitaminosis, clear cut examples of the therapeutic value of sunlight per se are conspicuously lacking. This statement should not be construed as a denial of the cures observed when sunbathing is included as a part of a regime used in the treatment of certain conditions, but only that the actual rôle of sunlight therein is not established beyond question. Moreover, one cannot completely disregard the numerous other claims for the therapeutic value of sunlight or ultraviolet radiation from other sources, particularly when one considers the success of the Finsen treatment of Lupus vulgaris[6].

In evaluating claims for curative effects of ultraviolet radiation, some papers dealing with prevention of the common cold merit discussion. Maughan and Smiley (184) reported 40 per cent reduction in the incidence of colds among subjects who received exposures to radiation from a mercury arc, as compared to control subjects who received no treatment. On the other hand Colebrook (65) and Doull, Hardy, Clark, and Herman (78) found very slight differences between exposed and control groups; the differences are probably not significant but both show fewer colds in the control than in the treated groups. Here are three studies, one of which indicates a significantly beneficial effect of ultraviolet radiation on colds, and two which show a slight detrimental, or probably no effect. The reason for the discrepancy may be sought in the mode of treatment, or in the criteria used for determining the incidence of colds. While the treatment was not identical in the three cases, one finds nothing here to account for the radical difference in the results. The criteria for determining incidence of colds are radically d fferent, however, Maughan and Smiley having accepted the reports of the subjects themselves, whereas Colebrook, and Doull, et al. depended upon objective examination. Diehl, et al. (73, 74) made extensive studies of the effect of various drugs and of a placebo (inactive medicament) on the incidence of colds, using the subject's report as a criterion, and found that the placebo gave approximately the same decrease in incidence that was observed by Maughan and Smiley after treatment with mercury arc radiation. Thus there is reason to believe that the decrease in the incidence of colds which Maughan and Smiley reported was a "placebo effect"[7], and that had a placebo

[6] The council for Physical Therapy of the American Medical Association (67) recognizes ultraviolet radiation as of possible value in the treatment of only a very limited number of conditions.

[7] i.e., subjective improvement following the administration of an inactive medicament or treatment.

been given to the control group, no difference in the incidence would have been observed. It may be mentioned that Colebrook used a very appropriate placebo in her studies, i.e., the same radiation filtered through glass to remove the wavelengths shorter than 0.32μ, and that she found no important difference between control, placebo, and treated groups under the conditions of her experiment. After considering this example, one cannot help wondering whether many of the other observed cures attributed to ultraviolet radiation are not of the same nature, for the widespread belief in its curative value renders it capable of exerting a strong "placebo" effect.

Bathing in the sunlight gives a pleasurable sensation of warmth when the ambient temperature is not too high or too low, and might be of value in convalescence, even though there is no specific effect of the sunlight. The pleasurable sensation no doubt comes from warming of the skin, and should not be greatly reduced by interposition of window glass, which at the same time would preclude the danger of severe sunburn.

Heat stroke. An extended discussion of heat stroke would not be possible in this review, but cannot escape mention since the solar heat load may be an important part of the total heat load, and hence may play a part in this syndrome, at times. Presumably hyperthermia is the direct cause in many instances (75, 261). Recent studies by Adolph and his collaborators (5, 6) indicate that under conditions of excessive dehydration, circulatory collapse finally results from reduction of the volume of the circulating blood to a dangerous level. In hot environments dehydration occurs from sweating if enough water is not imbibed to prevent the blood volume reaching a dangerously low level.

The vasodilatation that occurs in the skin as a result of sunburn could be an exacerbating factor in cases of heat stroke involving exposure to the sun, since it should act in the same way as reduction of blood volume, and hence contribute to circulatory collapse. On the other hand, the superficial vasodilatation might result in greater heat loss by radiation. The importance of these factors is difficult to assess.

It is possible that there are some direct specific effects of sunlight in heat stroke, but such have not been demonstrated. It has been thought that sunlight impinging directly upon the head may raise the temperature of the meninges and brain, because hemorrhages have been found in these structures after death associated with excessive exposure to sunlight (82, 241, 247). Experiments carried out by Aron (12) in the Philippine Islands do not support this belief, however. This investigator found that monkeys fully exposed to bright sunlight succumbed in one to two hours, hemorrhages of the brain being found at autopsy, but that if only the head was exposed the animals survived long periods of insolation. Apparently the total heat load was the lethal factor rather than heating of the cranium. Failure of the mechanism of heat control, e.g., due to a neurological lesion, might predispose to heat stroke, and this would be most likely to occur when the subject was exposed to an unusual heat load, to which sunlight might be a contributing factor. In such a case, sunlight could easily be credited with a specific rôle it does not play. Until further evidence of specific

effects is forthcoming, it seems necessary to conclude that "sunstroke" is not essentially different from heat stroke.

Sunlight as a Limiting Environmental Factor

Sunlight is a major factorin man's environment. It not only influences the ambient temperature, but is directly responsible for the winds, the major ocean currents and rainfall, besides being the source of energy for all life processes. Thus, sunlight largely determines the areas of the earth that are habitable; but for the most part indirectly through the above functions—not by direct physiological action. Nevertheless, a great deal has been written about the deleterious effects of excessive sunlight in the tropics, and of the lack of sunlight in high latitudes, and it is in order, here, to consider how the direct physiological effects of sunlight might favor or hinder the inhabitation of various parts of the earth.

Although the solar heat load may be an important part of the total heat load, it need not affect life in tropical countries if habits are properly adjusted. The wearing of white clothing can considerably reduce the solar heat load (39)[8], and outdoor exposure at midday can be avoided under ordinary conditions. Escape from the solar heat load should be an easier matter in the tropics than escape from the excessive heat load in some industrial plants, e.g., steel mills, in temperate climates (75, 128). Actually the intensity of the total radiant energy of sunlight, and hence the solar heat load, does not vary nearly as much with latitude as is commonly supposed. For example, reference to figure 1 will show that the difference in intensity with the sun at zenith (air mass 1), and with the sun at 60° from zenith (air mass 2) is not very great, amounting to about 20 per cent. With the sun at the summer solstice for the northern hemisphere, the intensity of the direct noon sunlight at the arctic circle is only a few per cent less that at the equator, At the north pole the total amount of sunlight received during 24 hours is greater at this time than at the equator because of the long summer day. Very hot days may occur in the arctic (e.g., 246), but the continuous recurrence of hot days and nights in the tropics no doubt constitutes a physiological and psychological stress which relatively short hot periods in the arctic do not achieve.

In contrast to the total intensity of sunlight, the intensity of the sunburn-producing component does vary greatly with latitude, due to the fact that the atmosphere absorbs strongly those wavelengths shorter than 0.32μ and hence the amount transmitted by the atmosphere varies greatly with the thickness of the atmospheric layer that must be traversed (see p. 505). The principal absorbing component of the atmosphere is ozone which varies in its distribution with respect to latitude and season; being present in least amount over the tropics where it remains approximately constant throughout the year, whereas in temperate latitudes it is present in greatest quantity during the winter months. This distribution of ozone tends to increase latitude and seasonal differences in

[8] The problem of proper clothing for hot environments is a complex one, involving numerous factors other than the reflection of solar radiation, e.g., see (261).

the sunburn-producing component of sunlight. The marked difference in this component of sunlight could conceivably be a factor in determining the inhabitability of the tropics as compared to temperate or arctic regions, but that it is an important one seems improbable. The discomforts and dangers of sunburn, while not a factor to be disregarded, can be avoided by simple precautions under ordinary conditions, although under military conditions this may be more difficult at times. It seems probable that more persons are severely sunburned in regions outside the tropics than in them, for inadvertent overexposure seems more likely to occur when the temperature is low, because of failure to realize the extent of the exposure. The potential danger of developing cancer of the skin may be greatest in tropical regions for reasons previously discussed, and this has been represented as an argument against inhabitation of the tropics by fair skinned races, but while it seems a hazard that should be guarded against, it also seems unlikely that the increase in incidence of skin cancer in the tropics over that in temperate latitudes would affect enough of the population to serve as a real deterrent to colonization (see p. 508). The danger could be largely avoided, of course, if it were recognized.

The antirachitic power of sunlight, since it results only from wave-lengths shorter than 0.32μ, must vary greatly with latitude, but deficiency in vitamin D can be so easily supplied in the diet, or by direct administration of the vitamin, that it can hardly constitute a factor limiting modern colonization in any climate. There seems to be no good evidence that life in complete darkness affects the physiology of man to any great extent (162), provided his diet is adequate, and there seems likewise to be little evidence that the arctic period of total darkness imposes any important physiological effects due to the lack of sunlight *per se*.

It has been frequently alleged that the negro is better adapted to life in the tropics because his pigment protects him against sunlight. This is an old belief (e.g., 70, 142) which seems to stem from a misinterpretation of various observations, and to the teleological point of view with which the matter has commonly been approached. The following quotation from Perthes who more recently championed this idea (217) typifies the teleological attitude, "Wenn es nicht nützlich wäre, so wäre es nicht, phlegte von veilen Jahren der Physiologe Pflüger seinen Schulern bei der Erörterung dunkler Vorgange im menschlichen Körper zu sagen".

One function that has been attributed to the negro's pigment is protection against the ingress of heat, but a glance at table 1 will show that a naked negro is actually at a disadvantage as regards the solar heat load because his skin reflects less of the incident solar radiation than does that of a naked white-skinned individual. This additional heat the negro must get rid of by evaporation, which means that he must drink more water and produce more sweat under like conditions of exposure than must the white man. It has been suggested that the pigment of the skin serves to raise the surface temperature when exposed to sunlight, and thus to increase sweating which in turn helps to dissipate the heat load; but measurements of skin-temperatures (153, 163) do not indicate

that pigment has any great influence on the superficial temperature gradient, and, moreover, sweating depends largely on internal rather than superficial temperature (160). Whatever the effect of pigment on superficial temperature the naked negro must be at a thermodynamic disadvantage as compared to the white man, since he absorbs more radiant energy; but any advantage or disadvantage in this respect would be virtually wiped out if the white man and the negro were both covered with the same kind of clothing.

The negro is less sensitive to sunburn and to cancer of the skin than is the white, and it is probable that the presence of considerable melanin pigment distributed throughout his epidermis is an important factor in determining both; but the advantage of this in competitive life in the tropics would appear to be relatively slight. As has been pointed out, sunburn can easily be avoided, and the proportion of cutaneous cancer among the population as a whole is small in any case.

Whatever the factors that account for the frequent success of the negro in supplanting the white man in the tropics, so carefully considered by Price (220), it seems most probable that the direct effects of sunlight have no great influence. On the other hand, it appears likely that the prolonged effects of constant hot environment, about the physiology of which we are not well informed, is an important factor in the "degeneration" of the white man in the tropics, but this is not a direct effect of sunlight.

Man now having added a third dimension to the "habitable" earth, one must consider the physiological effects of sunlight at altitudes as high as airplanes can operate. The increase in total solar radiation in going from the earth's surface to the outside of the atmosphere is not a very important factor, amounting to only about 35 per cent, as may be seen by comparing the curves for air mass 0 and air mass 1 in figure 1. The effect of the cold surrounding air and greater radiation to the heavens, makes cold rather than heat the important problem in high altitude flying. On the other hand the sunburn-producing ultraviolet radiation varies with altitude in a quite different manner and to a different extent; the ozone of the atmosphere, which is the component most important in cutting out this radiation, is stratified in its distribution, occurring in greatest concentration in the stratosphere. A number of measurements of the intensity of ultraviolet radiation at various altitudes indicate a considerable increase in the sunburn producing portion, beginning rather close to the earth (62, 76, 222); but the most complete measurements yet obtained, taken by O'Brien and his collaborators (206, 208) in connection with the stratosphere flight of the "Explorer II", indicates no appreciable ozone below about 15 kilometers, a height above the present limit of airplane operation. Whether or not there is an important increase in the incidence of sunburn-producing-radiation at present flying altitudes, and whatever the possibilities that the ceiling may be raised in the future, adequate protection should be readily obtainable by the use of plastics which do not transmit these wavelengths, or of window glass, in transparent enclosure surfaces.

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THE PRESENT STATUS OF THE PROBLEM OF THERMAL BURNS

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The problem of themal burns in its various aspects, physiologic and therapeutic, was reviewed in a mongraph by the author in 1942 which included the bulk of the pertinent literature up to December 1941. The present summary will cover the period 1942–1944 inclusive, except where previous writings have attained an increased importance or are subject to an altered interpretation in the light of recent developments.

Because some of the readers of this article may never have actually treated burns, the following composite case history can serve as a practical introduction to the review. In this case history certain of the physiologic problems, difficult or even completely unsolved, will be cited. The treatment used is generally adopted at the present time (January, 1945) and is an example of the present status of the treatment of burns.

Composite case history. A man aged 30 years was admitted with flame burns of the arms, chest, upper abdomen and face. The accident occurred one hour before admission from a gasoline explosion. At the site of the accident the patient was wrapped in clean towels and brought to the hospital. On admission he moaned but when questioned stated that he suffered little pain. One-quarter grain of morphine was given intravenously in the fluid which was started in a foot vein. Before starting this fluid, a specimen of blood taken from the same vein showed a hematocrit of 50. The fluid given was the so-called physiologic electrolyte solution (a mixture of two parts of normal isotonic—1/6th molar; 0.9 per cent—saline solution with one part of normal isotonic—1/6th molar; 1.75 per cent—sdoium lactate solution. The final mixture was of the following concentration: sodium chloride—1/9th molar; 0.6 per cent; 100 mM/L—and sodium lactate—1/18th molar; 0.6 per cent; 50 mM/L). While the physiologic electrolyte solution was running in, 500 cc. plasma was obtained and added to the infusion fluid. No shock was present.

At the same time as the above listed general treatment was begun, definite local treatment was instituted. All those in the room with the patient wore masks and caps and those directly in attendance wore in addition sterile gowns and gloves. The burned clothes were cut away, the burn estimated by Berkow's formula to be 32 per cent of the body surface, and a very minimal debridement was done with no additional anesthesia. Dirt was wiped away with soft cotton sponges using saline. No blisters were punctured and the entire burned area was covered with a four layer pressure dressing (1st layer: vaseline gauze; 2nd layer: sterile surgical squares; 3rd layer: large amounts of mechanic's waste; and 4th layer: elastic bandage or adhesive tape). This dressing was then left in place with no change until the fourteenth day.

The patient was sent to the ward and started on a routine of 200 cc. physiologic electrolyte solution by mouth every hour on the hour. The hematocrit was redetermined four hours after the accident (3 hrs. after admission) and found to be 57 despite the plasma and electrolyte previously given, so 1000 cc. additional plasma was ordered. (Such a continued rise in the hematocrit despite minimal parenteral therapy is common and indicates the need for continued blood concentration determinations.) Sulfadiazine was given by mouth. During the first 24 hours the patient received 2000 cc. plasma, 1000 cc. whole blood, and 1500 cc. of physiologic electrolyte solution intravenously and 4200 cc. physiologic electrolyte solution by mouth. During the second 24 hour period no plasma was given but 500 cc.

whole blood, 1000 cc. physiologic electrolyte solution and 500 cc. 10 per cent glucose were given intravenously while oral intake included 3400 cc. physiologic electrolyte solution, fruit juices and water ad libitum, and the first attempts at a high caloric, high protein diet. The shock phase (1st 48 hrs.) was thus passed without incident and the hematocrit did not arise above the reading of 59 obtained at the fourth hour.

The patient now entered the so-called toxemic phase (48–120 hrs., occasionally as late as the third week). Physiologic electrolyte solution, 2400 cc. per day, was kept up until on the fourth day a mild alkalosis (CO_2 combining power of 70) caused a shift to be made to normal saline solution. (The eventuality of acidosis could be met by a shift to isotonic one-sixth molar sodium lactate solution by mouth.) Careful attention was paid to the urinary output and none of the other signs of burn toxemia (jaundice, stupor, delirium) developed. On the sixth day the hematocrit had decreased to 39 so more attention was paid to the high protein diet and several whole blood transfusions were given.

After the shock phase was over and coincident with the toxemic phase, the patient entered the so-called septic phase. Sterile early definitive treatment plus oral sulfonamides, penicillin, maintenance of nutrition, and infrequent dressings, using mask technic, are the chief means of combatting such a condition.

The need for control of the phase of burn anemia and hypoproteinemia was especially accentuated when the first dressing of the burn was done on the fourteenth day. It was found that much (18 per cent of the body surface) of the burn was third degree and in the preparation for the inevitable and all-important skin grafting additional protein and hemoglobin were continuously lost from the exposed oozing surface. In places, granulations existed and in others sloughs which had to separate before the granulations would be ready for grafting. Infection, particularly with streptococci and pyocyaneus organisms, was present, especially where the sloughs existed.

Grafting was begun on the 32nd day and completed by the 56th. During this time strict attention had to be paid to a high protein, high vitamin diet (as much as 300 grams protein per day), and to occasional whole blood transfusions to keep the plasma proteins above 6.0 grams/100 cc. and the hemoglobin above 80 per cent. Once the grafted areas were entirely healed, fever, anemia, and hypoproteinemia disappeared promptly despite relaxation of control measures.

Several months later, when the patient was in good general shape, he returned for plastic improvement of one badly scarred healed area. This was done by substituting a thick graft for the thin scarred one already present.

In the remainder of this paper, various questions raised by the composite case report are presented under 23 headings as follows:

I. The Local Burn Lesion.

1. Immediate trauma (1)
   a. Necrosis (2)
   b. Edema (3)
      Compression treatment (4)
   c. Increased lymphatic flow (5)
   d. Infection (6)
      Rationale of early cleansing (7)
2. Determination of the area and depth of the burn (8)
3. Separation of burn sloughs (9)
4. Healing and regeneration (10)

II. The General Disturbances of the Burned Patient.

1. Morphine and anesthesia (11)

2. Burn shock (12)
   Plasma loss and plasma therapy (13)
   Burn shock complicated by pulmonary damage (14)
3. Importance of environmental temperature in influencing the mortality following severe burns (15)
4. Burn toxemia (16)
   The liver lesion and tannic acid (17)
5. Sodium therapy (18)
6. Burn anemia (19)
7. Burn hypoproteinemia (20)
   Nutritional factors (21)
8. Metabolic factors (22)
9. Endocrine relationships (23)

Several of these items were more or less adequately covered by the previous review (1942). Others (especially nos. 4, 5, 7, 14, 18, 20, 21, 22, and 23) were not so satisfactorily presented in the 1942 book because of new developments since that time.

I. *The Local Burn Lesion.* 1. *Immediate trauma.* Immediate trauma is important in burns. Locally it produces necrotic and inflammatory changes and may injure certain constituents of the blood flowing through and beneath the burned area. In some instances the blood may be hemolyzed and in others it may be heated to such a degree that generalized hyperthemia results. In still other cases the respiratory tract may be directly injured by heat or fumes. When severe enough, the immediate trauma causes death. Most such cases never reach a hospital and in the mortality statistics they are listed as victims of conflagration. Other patients, more fortunate in that their immediate trauma is less severe and their resultant burns less extensive, live to go through the various phases discussed below. In such instances certain of the local effects of the burn are as follows:

2. *Necrosis.* All deep burns are accompanied by actual destruction of tissue and it is this very fact which makes all forms of treatment imperfect. No method of therapy can restore the damaged cells to normal. The importance of the depth of the burn is recognized by the various classifications of severity of burns according to the penetration of the injury (Dupuytren, Boyer, Morton, etc., reviewed by the author, 1942). The reports of Elman and Lischer (1944); Leach, Peters, and Rossiter (1943); and of Prinzmetal, Bergman, and Hechter (1944) are of especial interest in this regard and consider the subject from a new standpoint. The St. Louis workers found three grades of damage to the skin: edema (produced by low temperatures or short exposure), wet necrosis (produced by medium temperatures and more exposure), and dry necrosis (produced by higher temperatures and more exposure). These authors pointed out that the type of necrosis may be more important than its depth. Their observations were made on anesthetized animals but were correlated with clinical observations. They finally presented evidence that wet necrosis, even though a less severe

injury than dry necrosis, may be more apt to lead to toxic and septic changes than the latter. Cellular infiltration, edema, and other signs of inflammation were noted in the wet necrosis type of lesion and not in the dry necrosis.

The Oxford group of Leach, Peters, and Rossiter (1943) in an excellent paper also considered the effect of the depth of the burn on general bodily changes. With a special water heated burning iron, these authors applied graded temperatures of 45 to 80° C. to shaved anesthetized guinea pigs, and in some cases rats, for times varying from 10 seconds to 6 and 10 minutes. Application of 47°C. up to 6 minutes produced no visible change. At 50° to 55°C., applied for 1 minute and over, there was a critical temperature for the development of permanent and irreversible damage; in animals good scab formation occurred after burning at this temperature. After temperatures of 60° to 65°C. the epidermis could be peeled off from the exposed area, leaving a punched-out exposed surface area somewhat like the exposed human blister. A temperature of 70° to 80°C. for 10 to 20 seconds produced severe scabbing. Observations of edema formation were checked by wet and dry weights of specimens of skin taken 2 hours after heating. At 55°C. there was some change, but this was more definite at 60°C. Two different types of histologic reaction were described. With milder burns there was cellular disintegration; with more intense burns, heat coagulation. In more intense burns there was a peripheral shell of changes characteristic of burns of lower temperatures. Two substances were apparently lost by the epithelium of the burned skin, namely (a) basophil granules from the cytoplasm, and (b) nucleoprotein from the nuclei. These two substances could be identified in the intercellular blister spaces. Finally Leach and his associates found that collagen fibers change in structure and staining affinity in the more intense burns.

Prinzmetal and his associates in Los Angeles also reported experiments indicating different effects from mild and severe burns.

1. Rats which were mildly burned at 75°C. for ten seconds on a single hind limb developed marked visible edema with considerable local fluid loss but did not develop shock.

2. Rats whose single hind limbs were severely burned at 100°C. for from two to three minutes died in shock without visible edema and exhibited insufficient local fluid loss to account for death. Similar results were obtained when larger areas of the body were severely burned for ten seconds.

These authors concluded: "It is therefore apparent that there are at least two mechanisms capable of producing shock; one due to local fluid loss and the other due to some unknown factor(s)."

Ham (1944) reported that in more severe second degree burns the skin surface tended to be white rather than red. Microscopically this was seen to be due to the superficial set of vessels being coagulated. Plasma loss in this type of burn originated from deeper capillaries. In this connection, Ham pointed out that the substance of the skin of both man and the hog is not evenly supplied with capillaries. He found that, in burns severe enough to seal off the superficial set, the next important set to become involved was that associated with the

deepest portions of the hair follicles and the deep secreting portions of sweat glands. These become dilated and congested and leak plasma. The leaking plasma, moreover, escaped from the skin down into the subcutaneous tissue. In more severe burns a third great capillary bed was affected, that of the subcutaneous tissue.

The possibility is to be considered that necrosis may result from trauma or treatment received after the original burn. Pressure dressings as a method of local therapy protect the burned area from mechanical or septic trauma. On the other hand, it has been shown by Taylor (1936), Cannon and Cope (1943), Hirshfeld, Pilling and Maun (1943) (on men), Maun, Schneider, Pilling and Hirshfeld (1943) (on dogs), and Clowes, Lund, and Levensen (1943) that other local remedies, tannic acid in particular, may increase the already existing necrosis of the skin and destroy remaining skin islands. Clowes, Lund, and Levensen (1943) studied 150 cases of burns from the point of view of comparing results following surface applications of ointment gauze dressings, tannic acid-silver nitrate, and triple dye. Areas treated with vaselined gauze became free of slough and were grafted earlier than areas treated with the other two methods. Dingwall and Andrus (1944) studied a controlled series of 82 second degree burns in human volunteers and included observations on the efficacy of 12 different local treatments. A sulfonamide-impregnated plastic film gave the best results, a bland ointment locally combined with sulfonamides by mouth gave the next best results, while "our experience in this series of experimental burns gives additional evidence for the abandonment of any type of treatment with escharotic agents."

3. *Edema.* All burns of second or third degree have accompanying edema. In fact the edema following thermal injuries is one of the most outstanding results of such trauma. Drinker (1944) stated in this regard: "Abnormal leakage from the capillaries is the dominant continuing characteristic of the burn lesion." In human beings the loss is both into the tissues and from the burned surface in the form of weeping. The amount of edema is considerable and was shown experimentally by Blalock (1931) to be sufficient to cause shock (i.e., 3.34 per cent of the body weight). The nature of the fluid lost is also of importance. Beard and Blalock (1931) (tissue edema fluid) and McIver (1933) (blister fluid) and others demonstrated that the exudate is plasma-like and not just a watery transudate. Presman, Janota, Weston, Levinson, and Necheles (1943) made simultaneous analyses of blister fluid and blood plasma. Blister fluid contained a concentration of proteins equivalent to 70 to 80 per cent of the plasma proteins The albumin content of blister fluid was fairly constant, but the globulin content showed decided variations independent of the albumin or globulin values of the blood and of the albumin values of the blister fluid. These results may be correlated with those of Lischer, Elman, and Davey (1944) who found that in experimental burns in dogs the serum albumin concentration tends to fall. In their experiments, the globulin fraction also fell in relatively less severe burns, but high levels were often noted in fatally burned animals or in those subjected to higher thermal stimuli. The rate of formation of the fluid

is rapid and it occurs largely during the first 18 hours and practically stops after the first 48 hours. (See item 13.)

With the rapid formation of large amounts of plasma-like fluid, the therapeutic implications would seem to be clear. The use of pressure dressings, of plaster casts or of cold (vide infra) represents an external means of controlling loss of fluid, while the recent paper of Berman, Peterson, and Butler (1944) introduces an internal method of accomplishing the same thing. These authors injected isotonic solution of sodium chloride hypodermically into the burned area in experimental anesthetized animals. Such an injection increases the hydrostatic pressure within the tissues and apparently in this manner minimizes plasma leakage. As a result of this treatment the survival time was considerably increased in animals so treated.

The edema of burns is associated with local vascular injury. As to whether or not there is a generalized increased capillary permeability considerable discussion still exists. Fine and Seligman (1944) injected plasma proteins tagged with radioactive iodine into dogs with burn shock. No evidence of leakage due to a change in the permeability of the generalized capillary bed was found. Tagged plasma proteins escaped into the area of injury in large amounts, but not into untraumatized areas. Cope and Moore (1944) studied capillary permeability in experimental burns using radioactive dyes. Following a hot water burn of a leg, the concentration of radioactive colloids in the lymph rose abruptly, indicating an increased capillary permeability to these substances. In only one experiment was there evidence of increased capillary permeability in an area remote from the burn. This single exception was an instance of late shock.

Netsky and Leiter (1943) used an immunologic method to study local and general capillary permeability in burn shock. The presence of horse serum after intravenous injection was tested turbidimetrically in the lymph. It was found that there was a prompt increase in the passage of protein, both in the burned and non-burned areas. This experiment thus gives results at variance with those listed in the preceding paragraph where the radioactive ion method was utilized.

Studies of the mesenteric vessels by the method of Knisely (1943) as used in burned cats by Abell and Page (1943) offers promise in the elucidation of the mechanism of burn edema and plasma loss. The work of Menkin (1943 and 1944) is also apropos. This author has isolated a substance from inflammatory exudates which shows irritating properties and causes leukopenia on injection. It does not depress the blood pressure of the cat. Menkin named this substance necrosin. It is antigenic and can induce a high precipitin antibody titer when injected into rabbits. An additional substance, leukotoxine, acts differently than necrosin in that it is leukocytosis-promoting, and is non-antigenic.

Evans and Hoover (1943) pointed out that in second degree burns most of the plasma loss is to the exterior in the form of weeping while in third degree burns it is chiefly into the tissues: "In our experience, there is practically *no* external loss of plasma in 3rd degree burns; the loss there is the 'white hemorrhage' of Allen and Koch, plasma lost into the subcutaneous tissues under the burn.

It is in the 3rd degree burn that one sees the poorest results when tannic acid is used. To us it seems wholly illogical to cover the surface of a 3rd degree burn with a heavy eschar to prevent plasma loss. The net result is no decrease in plasma loss." Several unsolved problems exist in connection with the edema of burns. One considers the fate of the fluid and is discussed below under item 5. The other relates to the possible toxicity of the fluid and will be discussed in connection with item 16. Ham (1944) also studied the edema in burns of different depths and its relation to the tannic acid method of treatment.

Hirshfeld, Williams, Abbott, Heller and Pilling (1944) point out that "3rd degree burns do not 'weep' significantly during the first 48 hours because the burned skin has so coagulated that it forms a firm leathery insensitive eschar. However, after a few days this eschar softens and begins to slough, allowing copious exudation which persists until skin-grafting has been completed."

4. *Compression treatment.* In 1924, Blair of St. Louis stated: "There are chiefly four basic things to be gained by the use of properly applied mechanical pressure to wounds: The elimination of dead spaces. The control of oozing. The limitation of venous and lymph stasis. Limitation of the amount of plastic material that pours into the wound." The method of pressure dressing treatment of wounds particularly used by plastic surgeons Blair, Davis, and others, was applied to burns by Koch of Chicago (1941) and soon adopted by others: Allen and Koch (1942), Siler and Reid (1942), Cope (1943), Evans and Hoover (1943), Koch (1943), Owens (1943), Harkins (1943), Gurd and Gerrie (1944), etc. Excellent reviews of this aspect of burns are given by the National Research Council (1943) and by Lund (1943). This method involves a compression dressing which is changed infrequently. Koch (1944) stated: "The essential features of such treatment are surgical cleanliness, compression of the injured surface to prevent fluid loss, and rest." Another means of giving uniform pressure without disturbing the wound is the use of rigid "theroseal" jackets as advised by Douglas (1944) by means of which hydrostatic pressure is applied.

The work of Burch and Winsor (1944) on the rôle of the skin and corneal layer in edema formation is somewhat apropos to this discussion. These authors showed that the dead, keratinous, even desquamating, thin, corneum acts as an elastic "pressure dressing" whenever edema forms. Possibly one of the causes of burn edema is that due to the thermal trauma this natural defensive mechanism breaks down. Roback and Ivy (1944) concluded from their experiments on dogs that "a firmly applied dressing decreases the time required for the complete epithelization of a burn wound."

Modern pressure dressings are usually of 4 layers: fine mesh gauze next to skin, surgical squares, loose fluff (mechanic's waste), and a compression roller. The nature of the first layer is the chief focus of debate. Everything from dry gauze or rayon to boric acid ointment (Cope, 1943) or sulfonamide permeated gauze is used (Harkins, 1943). Farr (1944) modified the inner layer of the occlusive dressing of burns by applying sulfanilamide ointment to the surface and then in turn covering this with transparent cellophane. Pressure was

applied to this and the method was used in 10 second degree burns with success. Hawn, Bering, Bailey, and Armstrong (1944) presented observations of surgically denuded areas of animals and of burns on humans on which fibrinogen and thrombin were used as a coating. No ill effects were noted and such films acted as a satisfactory dressing. The fibrinogen and thrombin were obtained as by-products of the processing of blood plasma at Harvard Medical School. The authors suggested that "such films, particularly in the form of roll bandages, might prove a highly expedient fibrinogen-thrombin dressing for burns in the field, owing to simplicity and speed from the standpoint of application, and to lack of bulk from the standpoint of transportation." This mode of treatment is somewhat similar to the use of human fibrin by Macfarlane (1943) in eight cases of accidental burns and two cases of experimental human burns.

The recent observations of Pfeiffer, Hallman, and Gersh (1944) on the toxicity of boric acid ointment used in the treatment of burns are of especial interest. These authors tested the ointment on dogs and found that "treatment of a burn involving only 4 per cent of the surface area of the body with U. S. P. 10 per cent [boric acid] ointment, will produce pathological changes in the central nervous system." These authors concluded as a result of their work: "Boric acid, whether applied in the form of an ointment or saturated solution to extensive wounds is a cumulative poison. The weak antiseptic value of boric acid suggests that for most uses, other more active and less potentially harmful therapeutic agents should be employed."

This work on the toxicity of boric acid repeats an old story in the history of burn therapy; one noted before in the case of picric acid and later of tannic acid treatment. This is that each time man attempts to become more elaborate and use anything other than the simplest of coverings on an extensive burned surface, he is apt to do more harm than good.

Another means of exerting pressure is to apply a rigid dressing before swelling has taken place and to allow the edema to exert its own pressure. This is the basis for the use of plaster casts as advocated by Glenn, Gilbert and Drinker (1943) (experimental), Levenson and Lund (1943) (clinical), Lund (1943), Sellers and Willard (1943), Barnes (1943), Sellers and Goranson (1944), and Glenn (1944) reported the use of plaster casts in 16 cases of infected burns of the extremities with good results. Most of these cases were treated in from 4 to 21 days after injury, only one being seen sooner (17 hrs.). Thus, this report is concerned essentially with a different problem than the primary treatment of burns. Elevation of the burned part, especially when plaster is used, was emphasized by Barnes (1943) as a means of reducing edema. Another physical method of reducing edema and plasm loss is the application of local cooling as done by Sellers and Willard (1943) and by Allen (1944). The principles which govern the treatment of burns with plaster casts as outlined by Barnes (Great Britain) (1943) are: "A simple technique of treatment, which includes the use of Plaster-of-Paris casts for burns of the hands and forearm, is described. If plaster is applied, elevation of the limb is essential, to prevent edema, and the hand must be placed in the correct 'position of function.' The value of immo-

bilization, infrequent dressing, and the prevention of edema is pointed out. Burnt hands do not become stiff merely as a result of immobilization, provided that this is carried out correctly for a limited period. In spite of the presence of infection with *Ps. pyocyanea*, skin grafts will 'take' on granulating burns that are dressed infrequently."

Glenn (United States) (1944) stated these same principles as follows: "At the present time these directions for using closed-plaster dressings in the treatment of burns may be followed. After minimal cleaning and debridement and without breaking blisters, two layers of sterile gauze are laid over the burned surface extending over the area to be covered by plaster. If fingers are involved they are enclosed separately in the gauze. A thin sheet of roller plaster bandage is then applied to the part without pressure, extending over the end of the extremity and two to four inches above the burn. In mild burns the encasement is removed in 7 to 14 days. In more severe burns it may be left in place for as long as four weeks. If the burn is incompletely healed or the epithelium is very thin, another encasement should be immediately reapplied. Grafting may be started as soon as the slough separates."

Lischer and Elman (1943) reported that in dogs the use of local elastic pressure after burns decreased the degree of hemoconcentration, but did not reduce the mortality. The importance of tissue pressure in the edema of burns was demonstrated by Bellis (1942) and Rossiter (1943). This may increase from 1–3 mm. Hg to 10–20 mm. Hg. Rossiter showed that pressure of the order of 10 mm. Hg applied externally will gradually lessen the edema in the skin of a burned guinea pig or rabbit. Another method of increasing tissue pressure is to inject saline solution into the burned area as was done by Berman, Peterson, and Butler (1944).

One of the purposes of the tannic acid treatment of burns is to prevent plasma loss. It manifestly does stop weeping from the burned surfaces, but its action on tissue edema is less certain. Ham (1944b) stated that his work "provides no evidence to suggest that the tannic acid treatment would be equally efficacious in the more severe second degree burns or in third degree burns where plasma loss occurs primarily from capillary beds out of reach of the tan."

5. *Increased lymphatic flow.* Lassar (1877) performed experiments in which a hind leg of animals was placed in water at 50 to 54°C. for 5 to 6 minutes with resultant marked swelling. He reported that there was an increased flow of lymph in scalded legs and believed that this was the mechanism whereby the fluid was reabsorbed and a return to normal effected. Wood (1940) tested lymph collected from the thoracic ducts of burned animals for vasodepressor activity by intra-arterial injection into albino rats and found no evidence for such activity.

Glenn, Peterson, and Drinker (1943) extended previous observations of Field, Drinker and White (1932) on the lymphatic flow from a scalded hind limb of anesthetized dogs. They found a significant increase in lymphatic flow following the infliction of thermal trauma. Subcutaneous injection of tissue extract in the region of the burn caused a coagulation of lymph, with stoppage of lymphatic

flow. Sections of such an injected burned foot showed definite fibrin formation while the non-injected burned foot showed practically no fibrin. In addition, a burned foot with injection of tissue extract tended to swell much more than a non-injected foot because "local swelling depends notably upon the degree to which coagulation of the exudate occurs with imprisonment of the water which leaks from the blood capillaries." Further evidence for this hypothesis is afforded by the observation that heparinization of the dogs to the extent that formation of fibrin was prevented caused a minimal swelling of the foot after thermal trauma. Glenn, Muus, and Drinker (1943) studied the lymphatic flow from a scalded leg of a calf and compared its amount and chemical composition with that from the opposite untraumatized limb. The results were interpreted on the basis of the following logical assumptions: "We assumed that lymph coming from the burned area would contain a higher concentration of substances resulting from tissue damage due to the burn than would lymph from another part of the body, or serum taken from the general circulation. On the other hand, substances of small molecular size which accumulate in the serum owing to impaired function of any of the organs would be present in the same concentration in the serum and in the lymph collected from different parts of the animal."

Their studies seemed to indicate no increase in capillary permeability in normal regions of the body distant from the burn. They concluded that the increase in urea and creatinine was consistent with the usual observations attending oliguria. In their opinion the increase in creatine was due to local damage, while the increase in amino acid was of uncertain origin. This observation concerning rise in amino acid in shock due to burn fits in with the observations of Harkins, Long and associates (1944). The rise in nonprotein nitrogen was just as great in the serum or lymph from an unburned leg as in the lymph from the burned leg and hence does not seem to arise primarily from the region of local trauma.

Perlmann, Glenn, and Kaufman (1943) described a new globulin fraction in lymph collected directly from the burned area in calves. Since lymphatic flow from the site of thermal trauma may act in two ways: to hasten local repair or to carry off possible noxious products, this globulin may represent a "toxin" but as yet no exact significance can be attached to this finding.

6. *Infection.* This remains one of the chief factors in burn mortality which resists all attempts at adequate prevention and treatment. Graham (1944) lists the control of infection as *the* unsolved problem of burns. Local application of sulfonamides, in powder form, at least, has been discarded. Tennison (1943) reported that sulfasuxidine could be locally applied without rapid systemic absorption, but Poth and Ross (1944) refute this statement with a large series of clinical studies. Oral sulfonamides are most generally used, while penicillin by various routes offers promises but has as yet not fulfilled all of them. One fact of practical importance remains, namely, that symbiotic growth of skin and respiratory tract organisms is far more serious than are the former alone on a burned surface. This is the rationale of masking of both the operator and, if

possible, the patient during major burn dressings and is an additional argument for the infrequent dressing of early burn wounds.

While local use of powdered sulfonamides is not popular at present, interest has arisen in attempts to control burn infection by sulfonamide ointments (Allen, Owens, Evans, and Dragstedt, 1942; Jenkins, Allen, Owens, Schafer, and Dragstedt, 1945; and Gurd, Ackman, Gerrie, and Pritchard, 1942), of sulfonamide sprays (Coloviras, West, and Armour, 1942) or of sulfonamide films or membranes (Pickrell, 1942; Clark, Strakosch, and Leven, 1942; Skinner and Waud, 1943; and Andrus, Nickel, and Schmelkes, 1943).

7. *Rationale of early cleansing.* As discussed under "compression treatment" above, Allen and Koch (1941), Siler and Reid (1942) and most advocates of this method recommended early cleansing of the burn wound with soft soap, water, and, occasionally, with fat solvents. At the time of the Cocoanut Grove disaster in 1942, on the recommendation of Cope, those burns treated at the Massachusetts General Hospital received all the elements of a good compression dressing except the usual preliminary cleansing. The results were good and this institution as well as several others have continued to omit such ablutions of the wound, but are extremely careful not to introduce additional infection including that from the respiratory tract. Of the three kinds of debridement of burns: soft cloths, harsh scrubbing brush, and scalpel excision with immediate skin grafting, the first is the one under discussion here. The second accomplishes too little to compensate for the added trauma and necessity for general anesthesia it entails. The third offers promise in selected cases, but their number will always remain small. Furthermore, because of lack of easy early diagnosis of burn depth, the embarrassing possibility of excising and skin grafting a second degree burn is always to be considered.

The no-debridement method has the following rationale according to Cannon (1944): "No debridement of the burned surfaces was done because evidence accumulated indicated that an increase in contamination of the wound resulted. Only by vigorous scrubbing of the surface could this contamination be reduced. However, the trauma of such scrubbing necessarily causes damage to viable cells and reduces the effectiveness of the important factor of tissue resistance in preventing invasive infection." A fine mesh gauze is placed on the burn without rupturing the blisters. This plan is justified by the absence of bacterial growth in cultures of blister fluid taken at intervals after the burn. The outer wall of intact, or even of ruptured blisters, acts as a protective membrane and may be considered as Nature's dressing for the ideal covering for a burn. Lund 1944 epitomizes this as follows: "The less often you dress a burn the faster it heals," and also states: "I have become such a therapeutic nihilist on the subject of chemical applications to the surface of burns, that, as some people know, we have given up any applications now except dry gauze, counting on rest, pressure with or without plaster, as creating as good conditions as we know of for the healing of a burn, and allowing those dressings to remain up to three and even four weeks."

8. *Determination of the area and depth of the burn.* The methods of Weidenfeld

(1902) and of Berkow (1924) form the basis for the calculation of the area involved by a burn. By breaking the body surface up into segments, this estimation becomes easier and the Berkow method is the standard used in hospitals throughout the world. Lund and Browder (1944) have adapted this method to patients of all ages, showing that the surfaces of three crucial areas of the body (head, thigh, and leg) can be considered to vary relatively with age, whereas the other areas may be considered to be of constant proportions. The importance of the proportionate surface area burned is that it may serve as a rough guide to treatment, especially as to the plasma needs during the first 24 hours (Harkins, 1943).

The depth of the burn is usually classified according to the listing of Boyer or Morton: First degree, redness; second degree, blistering; and third degree, eschar formation. A more practical classification is into superficial and deep, or into those which do not and those which do granulate. The classification of Converse and Robb-Smith (1944) (see table 1, below) is of interest in this regard. Since granulating burns of any considerable size always need skin grafting, the importance of this differentiation is manifest. Unfortunately, it is extremely difficult even for those with considerable experience to tell how deep a burn is when it is first seen. A favorable result following an early incorrect diagnosis of third degree burn is the basis for the empiric adoption of countless local burn remedies.

Dingwall (1943) observed burns after sodium fluorescein injection with ultraviolet light and a filter. This author found that third degree burns were blue-black, whereas second degree injuries appeared yellow-green, generally more intense in color than the surrounding skin. This method was tested successfully on third degree burns in experimental animals and on both second and third degree burns of patients. Patey and Scarff (1944) took sections from clinical burns and also from experimental burns of the skin of breasts produced immediately before removal for carcinoma. A standard technic for producing experimental burns in human skin was described. Histological examination of the shed blistered skin was found to be of value in the determination of the depth of damage and in the control of treatment. Such examination was especially valuable if it showed the plane of separation to be intraepithelial; it was of less value when it showed complete epithelial separation, as there was then no indication of the presence or absence of the underlying damage. The degree of dermal destruction could be assessed by differential staining of whole-thickness skin sections, but the taking of such sections on a large scale has practical objections. Finally, Patey and Scarff found that a modified Van Gieson's stain, when applied to the burned area of patients gave some useful naked-eye indications of the degree of dermal involvement.

9. *Separation of burn sloughs.* Once the acute phase of a third degree burn is over, an inactive period of waiting several weeks for the burn slough to separate is always necessary before skin grafting can be done. Such a wait seems useless, but on the other hand may be Nature's best method of handling the situation. At least skin grafting cannot be done on top of dead tissue. Attempts to hasten

the separation of the slough have been made by Connor and Harvey (1944). These authors have used pyruvic acid at a pH of 1.9 in 8 per cent cornstarch and found that the slough of experimental burns could be removed in 48 to 72 hours, so that early skin grafting could be employed at the end of that period. Cooper, Hodge, and Beard (1943) had previously used an enzymatic digest for the removal of dead tissue following burns. Howes (1943) noted that such digestion damaged living tissue in some instances, whereas Connor and Harvey (1944) stated that with a carefully adjusted pH, early slough removal "can be accomplished without significant injury to viable tissues."

These methods are reminiscent of the acetic acid method of burn treatment introduced by Hartwell (1917) and popularized by Dorrance and Bransfield (1922). This technic was considered to be a method of "chemical debridement."

10 *Healing and regeneration.* Glenn (1944) pointed out that there are four chief factors which interfere with burn hearling: (1) too active cleansing or scrubbing of the part at the time of initial debridement, (2) the application of harmful local therapy, (3) infection, and (4) inordinate swelling of the subcutaneous tissue with resultant stretching of the skin and anoxia of its cells, especially in poorly vascularized areas or in regions where the skin is very thin. As already stated under the discussion of "necrosis," certain local applications to burned areas may actually retard healing and regeneration following such injuries. Other applications are made to burned surfaces late in the course of the lesion to hasten epithelial regeneration. The work of Brush and Lam (1942) is a classic in this field and demonstrates that most such agents do more harm than good to wounds of non-thermal origin. Auerbach and Doljanski (1944) applied a saline extract of adult chicken heart and an alcoholic precipitate of this extract, as well as a saline extract of seven days old chicken embryos to experimental skin wounds in rats. No increase in healing time was noted as compared to control animals. Hamilton (1944) studied chemical burns and wounds and wounds in rabbits when treated with nickel pectinate, using bland agents, chiefly cod liver oil and foille as controls. This author found that nickel pectinate reduced the healing time 22 per cent, but did so by keeping down granulations rather than by stimulating epithelization. Smith and Livingston (1943, 1945) studied the effect of 17 agents on experimentally induced wounds and burns (1372 injuries and 878 controls). "Of all these agents, only the chlorophyll preparations consistently showed any statistically significant effect in accelerating the healing of both traumatic and thermal wounds." Williams and Bissell (1944) tested 11 substances on uniform-sized wounds of rats. These agents (nickel pectinate and chlorophyll were not included) had little effect and the authors concluded: "No definite benefit was derived from the use of any of these substances." Cope (1944) summarized his opinion on this subject by stating: "No substance is known which expedites the healing of the wound above that obtained under conditions of normal nutrition and absence of infection." The most recent mathematical studies of the rate of wound healing are those of Apperly (1944). Barclay, Cuthbertson, and Isaacs (1943) approached the subject of wound healing differently. Dried thyroid gland or

small doses of 2-4-α dinitrophenol, when fed to young adult male albino rats throughout the period of healing of circular skin wounds, caused a significant reduction in the near time of healing (11 per cent and 15 to 27 per cent respectively). These authors did not advise these drugs for clinical use.

Normal healing and regeneration is complicated by the constant presence of dead tissue plus infection. Interesting recent work is that of Fell and Danielli (1943). Small shallow burns were produced on rats anesthetized with ether with a soldering iron 1.5 mm. in diameter applied for about one second. Changes in the skin phosphomonoesterase were studied during the healing stage. Pooled scabs were analyzed and the phosphatase content was estimated in terms of milligrams of chemically determined phosphate split off in a standard time. In the shallow burns such values were especially high during the stage of regeneration (around the fifth day). Patey and Scarff (1944) studied healing after experimental burns. They conclude: There is no evidence for regeneration from the interpapillary processes of the skin (rete pegs) and from our observations it seems almost certain that the areas of regeneration formerly attributed to this cause are in reality derived from the ducts of follicles or glands."

Converse and Robb-Smith (1944) studied 469 skin graft donor sites and drew analogies between them and burns with respect to healing. They found that the quality of repair was roughly proportional to the rapidity of healing. During two years' service at the American Hospital in Britain (1940-1942) 191 burns were observed. The healing of these burn wounds was observed and 63 biopsy specimens were removed for histologic examination. As a result of these studies an anatomic terminology for classifying the depth of burns was used by these authors as shown in table 1. In deep burns where the full-thickness of skin has not been lost, healing occurs with considerable skin contraction and an invisible loss in skin surface is noted. This contraction is called "inter-island contraction" by Converse and Robb-Smith. In such cases, epithelial resurfacing originates from the individual epithelial islands formed from each hair follicle, sweat duct or cluster of sweat glands. Each epithelial island tries to join its neighbors not only by cellular division and migration but also by contraction. These authors believe it is logical to state that the longer epithelial healing takes to resurface a wound the more marked this inter-island contraction will be. In such cases a thick layer of relatively avascular inelastic fibrous tissue is laid down which is covered by an atrophic epithelium of poorer quality.

Converse and Robb-Smith believe that "inter-island-contraction" is more apt to occur when the skin of the area is loosly attached to the underlying structures, for example on the dorsum of the hand or on the eye-lids, the latter instance resulting in ectropion; or in the vicinity of joints where the contraction tends to interfere with normal motion. These authors further state that the newly healed smooth shiny tight skin with avascular and inelastic scar tissue is the enemy of serviceable repair. Palliative measures such as massage, ointments, and other physical methods have their place, but too much time may be wasted in using them, particularly in burns of the hands, and in most cases the best treatment is the complete replacement of such scars by thick skin grafts.

These authors state that skin grafting is indicated in superficial burns for two main reasons: (1) for the relief of skin deficiency, of tightness, following interisland contraction of deep thermal and mixed burns; particularly on the dorsum of the hand, around joints, for ectropion of the lids or distortion of the features of the face; (2) to replace skin of poor quality. Thin, shiny or keloid skin is poorly resistant to every day trauma. It tends to crack and ulcerate, even as a result of cold weather. Return of sensation is poor, and the appearance of the skin is often disfiguring.

II. *The General Disturbances of the Burned Patient.* From the standpoint of saving lives, these general effects of the thermal injury are of possibly greater importance than is the local burn lesion. The correlation between the two is of great importance and should always be considered. Reviews of this aspect of the subject are given by Lund (1943), Cope (1944), Lam (1944), and Harkins

TABLE 1

Classification of burns (Converse and Robb-Smith, 1944)

Superficial burns (partial skin loss)	*Epidermal burns:* Erythema Epithelial desquamation *Dermal burns:* Blistering Destruction of superficial layers of the dermis	Heal well
	Deep dermal burns: Destruction of the dermis down to the deep layers *Mixed burns:* Small areas of total skin loss alternate with areas of deep dermal burns	Heal slowly, with contraction; may require skin grafting
Deep burns (total skin loss)	Destruction of whole-thickness of the skin into, or beyond, the fat	Heal with difficulty, producing contractions and deformities. Skin-grafting the rule

(1943, 1944). Several pertinent problems in the management of the general disturbances of the burned patient will now be considered in order as follows:

11. *Morphine and anesthesia.* Before the war, most burns treated in Great Britain were anesthetized before applying local therapy, while in this country general anesthesia was used only rarely. At present, such general anesthesia is used even less often than before. Morphine is used as an analgesic and such preliminary cleansing which cannot be done without supplementing morphine is considered too traumatizing to be advisable. The only question which arises is as to how much and by what route the morphine should be given.

Beecher (1943, 1944) pointed out that burn patients are apt to be hyperactive or even manic from three causes: Pain, fear and hysteria, and cerebral anoxia. Morphine is a useful therapeutic agent only for those in the first of these three groups. In the other two groups it is not only ineffective but is contraindicated in large doses. When morphine is given subcutaneously to patients in shock it is apt to remain unabsorbed until shock therapy has been administered. After restoration of blood flow several doses of morphine might be absorbed

at once, with resultant collapse. Beecher points out that morphine should be administered intravenously to such patients. If because of the large number of cases to be cared for, one cannot take time for intravenous administration of morphine, the agent should be injected into an unburned extremity and the injection site marked with ink or dye so that if too great absorption results later on the inflow can be checked by means of a tourniquet.

Elman (1944) found in experimental burns that morphine increases the 24-hour mortality by as much as 100 per cent, particularly when given in large doses and with nembutal. He stated: "It is inferred that large doses of morphine when used in the absence of pain may increase the early mortality in severe burns."

12. *Burn shock.* Burn shock is chiefly a menace during the first 24 to 48 hours after the burn and is responsible for about two-thirds of burn deaths. While other mechanisms may play a rôle, the oligemia which results from plasma loss (item 13) is one of the chief factors. As a result of the oligemia, various cardiodynamic factors occur which are not primarily due to cardiac or vascular failure. It is true that cardiac damage does occur in burns (item 16), but essentially the heart and blood vessels are relatively unaffected, while the amount of blood they have to deal with is seriously depleted. Elman and Lischer (1944) summarized the importance of this latter factor by stating: "The production of inflammatory edema alone is clearly the cause of loss of plasma leading to hemoconcentration, fall of blood volume, and the physical changes responsible for surgical shock." The cardio-dynamic factors in burns include decreased cardiac output, diminished blood flow, peripheral vasoconstriction, intact vasomotor center, decreased venous pressure, and a late fall in arterial pressure. These items are all interrelated and in turn are all essentially secondary to the oligemia. Abell and Page (1943) studied the smaller blood vessels in anesthetized burned dogs and cats by direct vision. Vasoconstriction of the larger and smaller arteries and larger veins and constriction of arteriovenous anastomoses were noted.

It is somewhat uncertain as to whether a nervous factor exists in shock due to burns. Kabat and Hedin (1942) presented data of interest in this regard. Experiments were carried out on anesthetized cats, some of which had the spinal cord sectioned at L_1 or T_{11}. No appreciable difference was noted in the survival time of the two groups. The animals with the spinal cords sectioned showed much less hemoconcentration and local edema at the site of the burn than the control burned animals. These experiments seem to show that animals with transected cords behave differently after burns than do previously intact animals. They demonstrate that there is a nervous factor in burn shock, not that there is one in the causation of burn shock.

Adrenal cortical extract has been utilized in the treatment of burns. Rhoads, Wolff and Lee (1941) reported promising results from the use of this substance. They estimated that after a severe burn the capillaries do not regain their normal permeability until the fortieth hour, but after treatment with adrenal cortical extract such normality was attained by the eighteenth hour. A more recent report based on additional cases by Rhoads, Wolff, Saltonstall and Lee (1943)

reverses this opinion, stating that "the results of this larger series have not fulfilled the promise of the earlier cases. They do not provide a satisfactory basis for the use of adrenal cortical extract in the routine treatment of shock following burns." These clinical observations are corroborated by the experimental studies of Rosenthal (1943) who tested standardized burns in mice and found no protective action whatever by either desoxycorticosterone or adrenal cortical extract injected subcutaneously as compared with controls with saline solution.

13. *Plasma loss and plasma therapy.* Following a severe burn, various hematologic changes ensue. The early hemoconcentration following burns, first noted by Baraduc (1862), is so well known as to need no further comment. With the marked loss of plasma and resultant relative cell increase, there is a notable oligemia, possibly the most important of all the general changes in burns. There is apt to be a relative decrease in albumin and chloride, and a rise in potassium and other evidences of cell disintegration. The sedimentation rate is slowed early and increased later. The white blood count is greatly increased in the early stages and remains elevated if infection supervenes. The platelets may decrease markedly in number (MacDonald, Levenson, Davidson, Tagnon, and Taylor, 1944).

The possibility that the local edema and plasma loss could account for the hemoconcentration and burn shock was first postulated by Tappeiner (1881) and demonstrated quantitatively to be sufficient by Blalock (1931). The recent reviews of Blalock (1940) and of Rossiter (1943) are to be consulted in this connection. Rossiter concluded: "It is, therefore, probable that these enormous losses of plasma from the blood-stream will initiate shock. Even should this prove but one of a number of initiating factors, it is one of immense importance and one which justifies vigorous counter-measures."

These counter-measures include, especially, transfusion of plasma, albumin or whole blood of an amount calculated to fit the needs of the individual patient. The details of the treatment of burn shock were reviewed by Harkins, Lam, and Romence (1942), McClure and Lam (1943), and by Lundy, Adams and Seldon (1944). The use of gelatin in experimental burn shock was reported by Parkins, Koop, Riegel, Vars, and Lockwood (1943). Seven of the eight treated dogs died.

The need for plasma therapy has been recognized since Blalock's demonstration in 1931 that in fatal burns sufficient plasma is lost to produce death. Rossiter (1943) stated: "Shock is one of the most obvious clinical manifestations of severe burns. Evidence is given for the view that the plasma loss known to occur after severe burning is, in most cases, sufficient to initiate shock." Elman (1943) presents statistical data demonstrating the value of plasma transfusions. The time when such transfusions are essential is variously stated as the first 36 hours (Rhoads, Wolff, and Lee, 1941) and by inference from the hemoconcentration curves of Elman and Brown (1943), the first 14 hours. Few persons with burns of less than 10 per cent of the body surface burned will require plasma transfusions. Lombard (1943) reported on the use of plasma transfusions following burns as used in Africa.

Plasma dosage is estimated in three ways: extent of hemoconcentration, area of the burn, and clinical judgment.

If laboratory facilities are available, the following methods of estimating plasma dosage based on blood concentration may be used:

1. Formula of Black (1940).
2. Formula of Elkinton, Wolff, and Lee (1940).
3. More recent formulas (of author, 1941–1944 except where stated otherwise).

(a) Give 100 cc. of plasma for every point the hematocrit exceeds the normal of 45;

(b) Give 50 cc. of plasma for every point the hemoglobin exceeds the normal of 100;

(c) Give 300 cc. of plasma for every gram the hemoglobin exceeds the normal of 15 grams per 100 cc. (Phillips, 1944);

(d) Give 100 cc. of plasma for every 100,000 the red cell count exceeds the normal of 5,000,000 per mm^3.;

(e) Give 150 cc. of plasma for every specific gravity increase of 0.001 above the normal whole blood specific gravity of 1.060 (Phillips, 1944).

4. Nomogram of Jenkins, Schafer, and Owens (1943).

In children the amount of plasma to be given should be proportionately less according to body weight. Furthermore, the calculations do not indicate the amount of plasma that will be needed to manage the entire course of the burn. Just as in a diabetic patient when the blood sugar indicates that 30 units of insulin is necessary, this does not mean that 30 units will suffice for a whole month, so in a serious burn case frequent determinations are essential. Hematocrit readings should be taken as often as every three hours during the first day after a severe burn and the amount of plasma indicated each time given. The danger of burn shock is not over until the thirty-sixth to the forty-eighth hour.

Administration of plasma in proportion to the burned area is of value where laboratory facilities are available. Use of "first aid formula" (1942) involves the administration of 50 cc. of plasma for every per cent of the body surface burned by a deep (blistering) burn (see item 8). This amount should not be administered all at once, but according to the following schedule: one-third the first two hours, one-third the next four hours, and one-third the next six hours. Presman, Janota, Weston, Levinson, and Necheles (1943) do not believe that this formula gives quite enough plasma and suggest the following schedule: In the treatment of extensive burns in adults the immediate administration of 50 cc. of serum (or 60 cc. of plasma) for every per cent of body surface burned, and, in addition, 20 to 30 cc. for every per cent should be administered during the first twenty-four hours and another 10 to 30 cc. in the first seventy-two hours following the burn. Total administration, therefore, should be at least 100 to 110 cc. for each per cent of body burn. Others believe that 100 cc. for each per cent of the surface burned is too large a dose. In general, burns of the face, groin, or buttocks usually lose more plasma than the surface involvement indicates and more plasma should be given accordingly.

14. *Burn shock complicated by pulmonary damage.* The importance of pul-

monary damage in burns was first fully recognized by Wilson (1942). The incidence of such lesions was very high in the Cocoanut Grove disaster, and the resultant studies at the Massachusetts General Hospital led to the excellent group of articles by Beecher (1943), Aub, Pittman, and Brues (1943), Schatzki (1943), Mallory and Brickley (1943), and Cope and Rhinelander (1943).

Pneumonia may be of local or general causation or both. Thus, local damage due to inhalation of flames or hot air leads to pulmonary edema which may become secondarily infected. In certain instances pulmonary edema (see table 2) may also arise from a shocklike state. Walton (1944) stated in this regard: "Tracheotomies in those patients who have inhaled fumes are essential and lifesaving. If there is any question as to the need for this operation, it should be carried out without delay." This same author emphasized the general importance of sepsis in burns, stating: "Most of the patients with burns who die after a ten-day interval, die from sepsis." A summary of the present status of pulmonary edema in burns is given in table 2.

TABLE 2

Pulmonary edema in burns

Etiology. Pulmonary edema usually occurs from (a) inhalation of flames or very hot air or steam, (b) inhalation of noxious gases released at the time of the burn (e.g., Cocoanut Grove disaster), and (c) more rarely, as a part of generalized increase in capillary permeability or when there has been overtreatment with parenteral crystalloid solutions.
Time of onset: In fulminating cases, after several hours, occasionally, after two or three days.
Treatment:
1. Avoid excess fluids.
2. If coincident shock requires therapy, whole blood is best and plasma next best, while crystalloids may be dangerous.
3. Turn patient frequently.
4. Pressure breathing of oxygen.
5. Early tracheotomy in selected cases.

15. *Importance of environmental temperature in influencing the mortality following severe burns.* Before 1941, no advice concerning the treatment of burns was complete without mention of the so-called burn tent, usually kept at 85–110°F. In the light of present knowledge, such tents serve as perfect incubators for bacteria in the burn wound and markedly aggravate any shock which might be present. Since the report of Blalock (1941) concerning the dangers of excessive heat in shock, it is now recognized that all such tents are extremely harmful. Furthermore, with modern compression bandages, the patient is usually sufficiently insulated actually to benefit by a slight coolness of the surrounding temperature. This work has since been substantiated by Devine (1943) and others. Elman, Cox, Lischer, and Mueller (1942) studied the 24 hour mortality percentages of rats similarly burned by dipping into hot water at constant temperature of 80°C., but kept at four different temperatures afterwards. All animals placed in rooms at 32° and 99°F. were dead within 24 hours. At 55°F., 82 per cent died, while the lowest mortality of 26 per cent occurred when the animals

were kept at 75°F. This would seem, then, to be an optimum temperature, with increased mortality in colder or hotter environments, particularly the latter.

16. *Burn toxemia.* As Blalock noted in his recent monograph (1940) there is more evidence in favor of a toxic theory in burn shock than in most other forms. The problems of toxemia in burns were discussed by McClure and Lam (1940). One point favoring the toxemic theory is that the amount of shock present is not always sufficient to account for subsequent death. In fact, many burn patients die who have never had shock at all. Toxins afford a possible explanation for these deaths. Such supposed toxins may act directly on various cells or organs or may affect them indirectly by means of accompanying metabolic or endocrine disturbances. The relationship between the degree of skin damage and the possibility of toxemia has already been cited above in discussing the subject of necrosis (item 2) as an example of local pathologic change.

Rose and Browne (1942) made studies on the blood histamine, hemoglobin, hematocrit and plasma proteins following severe burns in seven patients. They found in certain cases a definite increase in blood histamine within one hour after the burn. In all cases, however, there was a decrease in blood histamine with the appearance of toxemia and edema. With the disappearance of these two noxious signs and with clinical improvement of the patient, the blood histamine rose to normal or higher levels. In their patients, such secondary increases in the blood histamine persisted above the normal for some time in certain instances. Rose and Browne noted a definite correlation between the degree of damage and the decrease in the histamine content of the blood. Thus, the greatest decrease in the blood histamine was observed 12 to 36 hours before death. These authors finally observed that the changes in the histamine content of the blood do not appear to be altered by the administration of plasma in amounts sufficient to control hemoconcentration.

The observations of Perlmann, Glenn, and Kaufman (1943) have already been cited in which they described a new globulin fraction in lymph collected directly from a burned extremity in calves. Antos, Dworkin, and Green (1944) studied shock associated with deep muscle burns. Such burns were produced aseptically in the hind legs of anesthetized dogs by means of strong diathermy currents. Five dogs so burned and restrained under anesthesia died with only moderate fluid loss as measured by a plethysmographic method. Five other dogs similarly burned but not restrained after burning survived and when killed showed marked fluid loss. These authors concluded that "The accumulation of fluid at the site of the burn is the principle factor responsible for the prodromal stage of shock in the burned dogs. Association of the extensive muscle destruction with restriction of movement and with prolonged anesthesia converts the prodromal stage into outspoken shock with death of the dog even when the accumulation of fluid at the site of the burn is quite small."

Anuria is one feature of serious burns which may be either a cause or a result of toxemia. Olson, Walker, and Necheles (1944) studied the urinary excretion in dogs burned under nembutal anesthesia. Anuria promptly resulted which did not always respond to massive doses of saline by intravenous injection. In

one experiment, despite a systolic blood pressure of 100 with anuria, a total of 2400 cc. of saline had no effect on kidney secretion. "The difference in response might be attributed to different amounts of hemoglobin in the plasma. . . . Permanent damage to the kidneys and anuria may develop following crushing or burning, because renal function seems to be most impaired following these two traumatic agents."

Miyata and Kayashima (1941) made clinical observations on patients with scalding burns. An early suppression of urine with gradual return to normal was noted. Urobilin and urobilinogen were demonstrated in the urine in about 50 per cent of the cases during the first week.

Kayashima (1940) reported electrocardiographic changes in patients and rabbits with burns. In 12 naval casualty cases (21–37 yrs.) in which 1/5 to 1/8 of the body surface suffered burns electrocardiograms which were taken denoted that the Q and S waves were deeper, the R wave lower and the T wave flatter than their respective normal waves, while the P-Q and Q-T values were almost unchanged. Clinically the patients had very small pulse pressures, and one showed absence of the sino-auricular contraction and three dominance of the left ventricles. In about half of the patients the dysfunction of the cardiac muscles was clearly noted on the electrocardiogram. With the improvement of the lesions the muscles showed a gradual recovery of their normal functions, but a certain amount of their functional impairment remained for a relatively long time. In further studies, Kayashima found that after experimental burns were inflicted on 10 rabbits the electrocardiograms showed changes in the waves, which were characteristic of the impairment of the cardiac muscles, noted in the electrocardiograms of patients suffering from burns. All the waves were low in amplitude; the T wave was flattened out and the ventricular complex tended to be monophasic. In 3 cases the excitation conduction was interfered with, producing extrasystole, auricular flutter and absence of the sino-auricular excitation. Histopathologically interstitial myocarditis with perivascular infiltration of the leucocytes was observed in 5 cases out of 7 autopsied. These results of Kayashima (1940) are to be correlated with those of Simonart (1938) who also worked on rabbits. It is of interest that Kayashima found flattened T-waves while in Simonart's experiments they were higher than normal. Histologic changes in the heart have been reported in human burn cases by Zinck (1938) and by Buis and Hartman (1941). In Simonart's experiments on rabbits, pathologic alterations in the heart included focal necrosis, calcification, some cellular infiltration, and proliferation of connective tissue. Fain (1939) and Gunther (1939) also noted pathologic changes in the heart following burns.

The question of the causation of Curling's ulcers following burns still remains uanswered. One thing is certain, however, that they are less common now than they were a hundred years ago, in 1842, when Curling saw 4 personal cases at the London Hospital in the 19 month period beginning September 1840. In a review of the literature, Harkins (1938) decided that the infectious theory was the most likely and the recent report of Hartman (1945) agreed that infection is at least one of the three chief etiologic factors. Verdan of Lausanne (1944)

reverted to the toxic theory reporting a case of a man aged 40 years with a gastric ulcer following burns associated with spinal cord damage. He postulated that toxins might have acted on the cord and this in turn caused the ulcer by a nervous mechanism. The case of fatal perforated esophagus occurring four days after a severe burn in a woman, aged 29, treated with tannic acid and reported by Robson (1943) may be somewhat similar in its origin to Curling's ulcer. The perforation was 2 inches above the cardiac end of the stomach and the probability of inhalation of flames was not present. Rankin (1945) also reported an esophageal perforation following a burn.

Kellaway and his associates (1943) have published results of burn experiments using perfusion of a heated limb. Previously, Nagamitu (1935) reported a vasodepressor substance in the fluid perfusing a burned limb. In the first paper, Kellaway and Rawlinson, 1944, studied the effects of heat on the isolated lungs of guinea pigs ventilated with oxygen, air and nitrogen. In these experiments the degree of injury produced was estimated by the amount of histamine set free in the perfusate and drainage fluid expressed as a percentage of the total content of the perfused tissue. The histamine assay was done using strips of isolated guinea pigs' jejunum suspended in an oxygenated bath of Tyrode's solution at 35°C. Torantil neutralization was used as an additional means of proving that the substance was histamine. The experiments indicated that ventilation with pure oxygen neither increases nor decreases the histamine output to any extent, while heating to 45°C. would produce an increase. These experiments essentially showed that in perfusion at least of the lung, anoxia does not cause gross injury and does not markedly aggravate heat injury in so far as histamine output is concerned. On the basis of these preliminary experiments the authors thought that they could give more credence to their subsequent ones mentioned below in which the factor of anoxia was not controlled. It can be said, therefore, that the liberation of histamine from the isolated lungs of the guinea pig is not significantly increased by anoxia more than that which attends the perfusion of an isolated organ with saline. The weakness in these experiments is that results obtained from the lung may not be applicable to those obtained from other organs.

In the second paper Rawlinson and Kellaway studied the liberation of enzymes from the perfused liver. The object of these studies was to ascertain the effects of cellular injury by moderate degrees of rise of temperature. Since they were especially interested in the liberation of enzymes, it seemed advisable before experimenting with complex structures such as limbs to study a relatively homogeneous tissue known to be rich in enzymes. They therefore perfused the isolated liver of the cat with saline at temperatures ranging from 38 to 50°C. Several substances were investigated. At 38°C. none of them reached a maximum output until at least 19 hours. A rapid increase in output of alkaline phosphatase, esterase, proteolytic enzymes, and to a lesser degree of histamine as the temperature approached 40–46°C. indicated that this is the critical level for heat injury. The maxima were reached in the following order: phosphatase, phosphate, proteolytic enzymes, esterase, and catalase. These time differences applied above 43° as well.

The Arrhenius equation in the form

$$\frac{d \ln k}{dT} = \frac{\mu}{RT^2}$$

involves the use of k as the rate constant of the process, T as the absolute temperature, R as the gas constant, and μ as an empirical temperature characteristic. Rawlinson and Kellaway used this as modified to

$$\log \frac{a}{t} = \frac{\mu}{2.303R}\left(\frac{1}{T} + C\right)$$

where a and C are constants and t is the specified time. This equation should be satisfied if a straight line is obtained by plotting $\log \frac{1}{t}$ against $\frac{1}{T}$. These authors assumed that for purposes of graphing at the temperatures studied, the degrees Centigrade could be substituted for the reciprocal of the absolute temperature. Thus, using $1 + \log \frac{1}{t}$ as ordinates and °C. as abscissae, an ascending essentially straight line was found within the ranges of temperature studied (38–50°C.) for the values of histamine and catalase. The alkaline phosphatase and esterase lines showed a marked rise from their previously straight course at about 46°C. This forms the basis for Rawlinson and Kellaway's assumption that plotting their values in this manner, there is a greater outpouring of these two substances from the perfused liver above 46°C. than would be accounted for by increased cell damage due to perfusion at a higher temperature.

Thus, the rapid increase in the return of liberation of such substances as inorganic phosphate, alkaline phosphatase, esterase, proteolytic enzymes, and to a lesser degree of histamine, when the temperature approaches 40 to 42°C. is considered to be greater than can be accounted for by the normal exponential increase of the processes operating at 38°C. On the other hand the liberation of catalase and histamine above 38°C. is slower than that of the other constituents studied. The liberation process for catalase satisfies the Arrhenius equation and it therefore may be concluded that the rate-determining steps are characteristic of the enzyme. The gross deviation from the Arrhenius equation by alkaline phosphatase and esterase above 44 to 45°C. demonstrates that inactivation is commencing. Catalase on the other hand shows no such destruction up to 50°C.

In the third paper, Kellaway and Rawlinson (1944) studied tissue injury by heat in isolated limb preparations. They believed that this offers a useful lead for the discovery of possible toxic products whether these be cell constituents or formed in the cells or tissue spaces by enzymatic action consequent upon heat injury. From the perfused hind limbs of the guinea pigs histamine was liberated from between 45° and 50°C. From the cat forelimb histamine was similarly liberated. In the early hours of perfusion a smooth muscular relaxing substance which inhibited the stimulating effect of histamine was present in the perfusate. No active adenyl compounds could be detected between 37.5° and 50°C., but

cardiodepressant activity was found between 42° and 50°C. This was present also in the subcutaneous edema and the substance was heat stable and was not diminished by incubation with cat muscle or cat liver extracts. The hydrogenized concentration of the perfusate showed greatest increase at 44 to 45°C. The small output of inorganic phosphates at 37.5° to 41°C. had superimposed upon it at the higher temperatures a much larger output. Alkaline phosphatase, lipase, and proteolytic enzymes were also set free above 41°C. Kellaway and Rawlinson concluded: "The demonstration that lipase and proteolytic enzymes were set free from the perfused hind limb of the cat above 41°C. gives color to the possibility that toxic products may be formed by enzyme activity in the tissue spaces."

17. *The liver lesion and tannic acid.* Early reports noted a central necrosis of the liver in fatal burn cases, but did not implicate tannic acid as the causative factor. These reports included those of Wilson, Macgregor and Stewart (1938); Belt (1939); Zinck (1941); Buis and Hartman (1941); Duffin (1942); Boyce (1942) and others. Previous observers, including Bardeen (1898), Weiskotten (1919), Weimann (1927), Vogt (1929), and Hiroto (1934) (all cited by Harkins, 1942), had reported liver necrosis in burns, but such a damage was more diffuse and was not the typical marked central necrosis with polymorphonuclear neutrophile infiltration associated with the tannic acid cases and first reported by Wilson, Macgregor, and Stewart (1938).

The possibility that tannic acid might cause focal central necrosis of the liver was first postulated by Wilson in Great Britain in 1938. Wells of Hartford, Conn. reported the first experimental evidence that tannic acid is the cause of liver necrosis in burns before a panel discussion at the Clinical Congress of the American College of Surgeons in Chicago, October, 1940. In a memorandum submitted to the Burns Subcommittee (British) in May 1942, Wilson referred to the possibility that absorption of tannic acid might be a cause of toxemia in human cases of burns so treated. His experience in the Middle East, though admittedly inconclusive, had indicated that such toxemia was more common and severe in burned patients treated with tannic acid, and he described definite pathologic changes in the livers of patients who had died in the toxemic stage after tannic acid treatment. This action was shown to be true experimentally by Wells, Humphrey and Coll (1942) and later by Forbes and Evans (1943), by Baker and Handler (1943) and by Hartman and Romence (1943). Erb, Morgan and Farmer (1943) studied the postmortem findings in 61 cases of burns. Of these, 41 were tanned, with 25 (61 per cent) instances of liver necrosis, and 20 were not tanned, with 0 (0 per cent) instances of liver necrosis. Rosenthal (1942) working with mice, found that "significant increases of early mortality were produced by . . . tannic acid solutions and ointment . . . when applied to a scalded area." These observations seem to point rather conclusively to the potential danger of liver necrosis from tannic acid therapy. A closer study of the reported cases and experiments indicates that the tanning methods in which the active agent is kept in contact with the burned surface in a moist state for a long period of time (tannic acid jelly or baths) are especially dangerous. The increased

chances for absorption seem to be the influencing factor, as pointed out by Harkins (1943) and Rae and Wilkinson (1944). It has long been known that acute toxemia may follow the wetting of a dry tannic acid eschar, and as Rae and Wilkinson state, this too may be due to absorption of tannic acid after wetting rather than to liberation of other toxins. Rapid tanning, as by tannic acid-silver nitrate, where the agents dry within a matter of minutes or seconds, is less dangerous in this regard.

Rae and Wilkinson (1944) studied the liver function after burns in children by means of the levulose tolerance test. Treatment included silver nitrate, tannic acid jelly, and sulfonamide. Disturbance of liver function was greatest with tannic acid jelly and least with silver nitrate. These authors stated: "There is now sufficient evidence, both clinical and experimental, to justify disuse of tannic acid as a local application for burns."

Wilson (1941) found that while injections of tannic acid were toxic to animals, silver nitrate in similar doses produced no such toxic effects. Cameron, Milton, and Allen (1943) produced the typical liver lesion by treating burned areas with tannic acid solutions. The degree of liver damage was related to the extent of injury and possibly also to its depth, since in goats, where the lesion corresponded roughly to a deep human burn, liver damage was less marked than in rabbits, where the injury was more superficial. Barnes and Rossiter (1943) obtained results somewhat similar to those of Cameron, et al. These Oxford authors found that when tannic acid and sodium tannate are injected intravenously, they are fatal to mice and guinea pigs in doses of the order of 40 mgm. per kgm. The fatal intramuscular dose was much greater. In rats sodium tannate given intramuscularly was more toxic than tannic acid. After application of tannic acid to the raw area produced by burning guinea pigs to the extent of a quarter of the body surface, there was slight but definite liver damage which was greater than that in the controls. Similar burns treated with tannic acid had a higher mortality than control burns of the same extent not so treated. In another paper, Clark and Rossiter (1943) studied liver function in rabbits after injection of tannic acid. After subcutaneous injection of 100 to 750 mgm. of the drug per kgm., there was a depressor of liver function as measured by the galactose-tolerance test. This impairment of function was also observed after the intravenous injection of smaller doses (5–10 mgm./kgm.) of tannic acid.

Cameron, Milton, and Allen (1943) concluded: "There seems little doubt that even with not very extensive skin burns the application of tannic acid may be followed by its appearance and persistence in the blood and the development of slight or moderate liver damage. So far we have not had any fatal results [experimental animals: goats and rabbits], but this is most likely a question of extent of burn and amount of tannic acid applied. In none of our experiments has the blood level of tannic acid been high; the period of time over which tannic acid persists in the blood seems to be much more important in the production of injury." These authors agree with Robinson and Graessle (1943) that these effects are not due to gallic acid.

Robinson and Graessle (1943) recorded experiments on the toxicity of tannic

acid. When applied to the deep subcutaneous tissue following surgical removal of the skin, tannic acid is lethal to mice, but not to rats or rabbits. Liver damage shown by increased retention of bromsulphalein resulted in rabbits from subcutaneous tannic acid administration but not when it was applied to denuded surfaces. Clark and Rossiter (1943) found that liver function, judged by galactose tolerance, is impaired more noticeably by subcutaneous than by intravenous injection of tannic acid, possibly because of long-continued absorption.

Other papers concerning the liver and burns include those of workers in Boston, Oxford, and Los Angeles. Muus and Hardenburgh (1944) (Boston) reported that the oxygen consumption of normal rat liver slices is increased when measured in lymph from the burned legs of calves or one dog as a medium. The Q_{O_2} in lymph after burning was as much as 41 per cent higher than that in normal lymph. A less noticeable increase was noted when serum collected after burning was used in place of lymph. The material which caused the increase in oxygen consumption was found to be present in the ultrafiltrate from the lymph after burning. In a later paper, Muus, Hardenbergh, and Drinker (1944) found that lymph from the burned legs of a series of dogs acted similarly, not only in increasing the oxygen consumption of rat liver slices but also of rat diaphragm muscle.

Clark and Rossiter (1944) of Oxford studied the metabolism of rabbit liver slices after burning. Anesthetized rabbits scalded over one-third of the body surface at 70°C. for 30 seconds demonstrated a hemoconcentration at the end of 4 hours which had disappeared in 24 hours. In these animals there was no change in the ability of the liver to oxidize alanine or sodium butyrate either 4 or 24 hours after burning, and there was no change in the $Q_G^{O_2}$ and Q_{O_2} measured in bicarbonate buffer. After 4 hours there was a fall in the R.Q. (measured in phosphate buffer), rise in the $Q_G^{N_2}$, and fall in the ability of the liver to form glycogen from glucose; these functions all returned to normal within 24 hours. After 24 hours there was a slight rise in the Q_{O_2} measured in phosphate buffer, this not being observed after 4 hours. These results are correlated with the time of onset and disappearance of hemoconcentration and it was concluded that they are probably secondary to the circulatory changes following the burn. Wilhelmi and Harkins (1944) also found that the Q_{O_2} does not change appreciably in rat liver slices over a period of 15 hours following a scale of a greater degree than that which Clark and Rossiter's rabbits received (rat scald: 80°C., 7 sec., over entire body surface except head).

The Los Angeles group working with Prinzmetal (Prinzmetal, Hechter, Margoles, and Feign, 1943 and 1944) reported that a principle obtained from liver is effective against shock due to burns, as tested on scalded etherized rats. Isotonic saline solution, while helpful, was not as active in preventing shock as was the liver action. The latter in turn was not identified with the antianemia principle. This work of Prinzmetal and associates is to be correlated with that of Forbes and McConnell (1937) on a liver faction protecting against liver necrosis from carbon tetrachloride or chloroform administration.

Abandonment of tannic acid has not entirely eliminated burn toxemia, and the

question still remains whether selected burn cases may not under certain conditions be treated with advantage by this type of therapy. A more complete discussion of this important subject is made by Lee and Rhoads (1944). These authors point out the disadvantages and advantages of the method and leave its future place open for subsequent opinion to decide.

McClure, Lam, and Romence (1944) wrote an article entitled: "Tannic acid and the treatment of burns: An obsequy." Coming from the same institution where the modern tannic acid treatment of burns had its birth in 1925, this report carried additional weight in indicting this mode of therapy. These authors stated: "Liver necrosis had been reported in a considerable number of burned patients treated with tannic acid. Nonfatal cases frequently showed marked disturbance of the liver function in the acute phase of the burn. The liver lesion is easily reproduced experimentally. Wound healing experiments on animals and on human donor sites indicate that tannic acid retards healing considerably." In conclusion, the words of Cameron, Milton, and Allen (1943) are a succinct expression of now popular opinion: "It seems that all may not be well with the tannic acid treatment of burns."

18. *Sodium therapy.* Plasma (or albumin) and whole blood is the accepted treatment of burn shock. Recently, however, various investigators have reported efficacious results with sodium salts, especially by mouth. Hoitink (1938) found normal saline solution to be more efficacious in hemorrhagic shock as a blood substitute than glucose or gum acacia and concluded that its intravenous application is "preferable to the use of other artificial blood substitutes and to blood transfusion."

Rosenthal (1943), experimenting on mice, produced a standard burn by immersing the animals up to the neck in water at 70°C. for a measured period of seconds. A large series of mice was thus burned and treated groups were compared with untreated controls as to mortality. Sodium chloride by mouth or intraperitoneally caused a significant reduction in mortality. Intravenous administration was less effective. Isotonic solution of sodium chloride by mouth was superior to hypertonic solutions Sodium acetate, succinate, bicarbonate and lactate were as effective as sodium chloride. Mouse serum injected intravenously was slightly less active than equivalent volumes of 0.9 per cent sodium chloride orally. These results are of great interest especially as they do not agree with the present concept of the superiority of intravenous plasma or serum in the therapy of burn shock. The possibility arises that the results obtained on mice are not entirely applicable to man.

Fox (1944) used oral sodium lactate in the treatment of 17 burn cases. Chilled one-sixth molar sodium lactate was given at frequent intervals so that approximately 7 to 10 liters (10–15 per cent of body weight) was given the first 24 hours. Any vomiting was treated by administration of more fluid. Fox reported good results in this series of cases and since the time of this report has used the method in a much larger series of cases (personal communication, Sept. 1944). Cope (1944) discussed the rationale of Fox's method.

Prinzmetal, Hechter, Margoles, and Feigen (1943) in working on mice and rats also reported good results with sodium therapy. They stated: "Nine-tenths per cent solution of sodium chloride, when administered [intraperitoneally] in amounts equivalent to 5 or 10 per cent of the body weight, is definitely effective against burn shock when given either after or thirty minutes prior to trauma." Parkins, Koop, Riegel, Vars and Lockwood (1943) studied burn shock in dogs and stated "it appeared that saline alone [intravenously] was beneficial to these animals." Levine, Huddlestun, Persky, and Soskin (1944) found in the so-called irreversible stage of hemorrhagic shock, whole blood supplemented with sodium bicarbonate and glucose (38 per cent mortality) was much better than whole blood alone (75 per cent mortality). This work implied that acidosis as well as a sodium deficiency may play a rôle. Coonse, Foisie, Robertson, and Aufranc (1935) also found alkali therapy to be of benefit in human traumatic and hemorrhagic shock and in experimental shock in dogs. On the other hand, Rosenthal (1943) found sodium chloride to be just as efficient in burn shock as is sodium lactate.

Moyer, Coller, Iob, Vaughn and Marty (1944) reported experiments on burn shock in dogs which tend to favor the view that acidosis may be a noxious factor in this syndrome. Their conclusions were: Saline-bicarbonate solution is more effective than Ringer's lactate solution, and Ringer's lactate solution is more effective than "isotonic" sodium chlorde solution in prolonging life of anesthetized dogs when 10 per cent of body weight of these solutions is given intravenously before the animals are scalded (two-thirds body surface at 85°C. for 30 sec.). The presence of the bicarbonate ion or the potential bicarbonate ion in the lactate solution appears to be responsible for the superiority of the Ringer's lactate and the saline-bicarbonate solutions. The combination of massive transfusions of defibrinated blood and saline-bicarbonate solution by stomach was the only form of therapy employed that prevented shock without inducing complications that were incompatible with life. The other forms of therapy employed, namely: (1) Saline-bicarbonate, i.v.; (2) saline-bicarbonate i.v. and serum i.v.; (3) serum i.v. and saline-bicarbonate by stomach; and (4) defibrinated blood i.v. and water *ad libitum*, prolonged life and in a number of instances prevented shock. But all the animals that did not die of shock during the first 24 hours died later of complications that seemed to be related to the therapy rather than to the trauma.

The work of Wilson, Macgregor and Stewart (1938) is of interest with regard to sodium therapy. These authors reported that a considerable fall in the level of serum sodium occurred in certain of their patients following extensive burns or scalds. Lowdon, McKail, Rae, Stewart and Wilson (1939) repeated this observation and also found that the serum sodium is rapidly restored to a normal level by desoxycorticosterone acetate. They then investigated these changes by animal experiments. Scalds were produced in cats under nembutal anesthesia by immersing the hind limbs and posterior third of the trunk in water at about 90°C. for 5 seconds. Subsequently the characteristic condition of "shock" supervened and the animals died in from 45 minutes to 4 hours after injury. After scalding, the level of serum sodium of arterial blood and of sodium in

cerebro-spinal fluid steadily declined, while the sodium in the red blood corpuscles tended to increase. These changes were not prevented by previous section of the spinal cord, decerebration, removal of the kidneys or of the suprarenal glands.

In control experiments without scalding the procedures of repeated withdrawal of blood samples, suprarenalectomy, nephrectomy, spinal cord section and decerebration produced no more than a small and transient fall of serum sodium. It seemed to these authors, therefore, that the loss of sodium from serum and cerebro-spinal fluid was independent of any action of the kidneys or central nervous system and that it was not due to impairment of functional activity of the suprarenal cortex, since the serum sodium did not fall after removal of the suprarenal glands in experiments of similar duration. It seemed logical to them to conclude that the diminished content of serum sodium was not merely a consequence of hemolysis of red cells which was an invariable sequel of scalding.

The following facts suggested to Lowdon and his associates that the sodium was being lost into the scalded tissues:

(1) No significant fall in serum sodium occurred if the circulation to the scalded area were effectively occluded before scalding.

(2) The serum of venous blood from scalded skin contained less sodium than did the serum of arterial blood.

(3) When the isolated hindquarters of the cat were perfused with heparinzed blood a sharp decline of plasma sodium began about 1 hour after scalding and the plasma of outflow blood had a significantly lower sodium content than the plasma of inflow blood.

They tested this theory by estimations of tissue sodium but the evidence was incomplete.

Lowdon, et al., next tested the action of desoxycorticosterone acetate in restoring the level of serum sodium after scalds in animal experiments; 45 minutes after intramuscular injection of desoxycorticosterone acetate the serum sodium had returned from a low to a normal level. How and from what source the sodium is mobilized was not determined.

Other changes in the blood were noted after scalding. Hemolysis of red cells has been mentioned; it precluded satisfactory estimations of serum potassium. The chlorides of serum (or plasma), like the sodium, diminished, but the chlorides of whole blood increased. A curious feature was that when, prior to scalding, the cat was decerebrated or the spinal cord sectioned, then the serum chlorides, but not the serum sodium, rose instead of falling. Urea and non-protein nitrogen increased both in serum and whole blood, and there was usually a moderate rise in corpuscular concentration. Finally, these authors found that substantially the same changes in blood chemistry which followed scalding were noted when shock was produced by hammering the thigh muscles of the cat.

The use of sodium therapy in burns is one of the chief points of interest at the present time. It would seem unwise, however, to discard the proven advantages of plasma treatment, and better to use the administration of sodium salts as an adjunct to plasma therapy rather than as a substitute for it.

19. *Burn anemia.* Most serious burns show a biphasic hemoglobin concentration curve. Early hemoconcentration is followed by a progressive anemia which begins to develop on about the fifth day and remains troublesome as long as granulating surfaces remain unhealed. Actually the anemia may have its onset at the time of the burn and the subject of "early masked anemia" was reviewed *in extenso* by Harkins (1942). Early in the course of the burn the erythron may be reduced, but such a diminished volume is concealed by the even greater decrease in the plasma volume. Actual red blood cell destruction with resultant hemoglobinemia and hemoglobinuria may occur at the time of the burn.

In an article describing a thrombocytopenia which occurred in 12 out of 13 burn cases in from 7 to 57 hours after the burn, MacDonald, Levenson, Davidson, Tagnon, and Taylor (1944) stated that "hemoglobinemia was present for varying periods of time in all of the 13 patients." Cope and Rhinelander (1943) reported 9 cases of hemoglobinuria in patients burned in the Cocoanut Grove disaster. Numerous older writers on the same subject including Lesser (1880), Pfeiffer (1905) and others are reviewed by Harkins (1942) and by Shen, Ham, and Fleming (1943). The latter article is one of the best presentations of this particular subject. These authors studied 14 cases of burns and found hemoglobinuria in 10 of them. Hemoglobinemia during the first 20–36 hours was present in 8 cases with values ranging from 65 to 215 mgm. of hemoglobin per 100 cc. of plasma. In 6 patients who had shown hemoglobinuria, pathologic examination of the kidneys showed hemoglobin casts. The osmotic fragility of the red cells was significantly increased above normal in 7 patients. In 4 patients tested the osmotic fragility of the red cells was increased in 3 when determined within a period of a half to 3 hours after the burn. Spherocytes were significantly increased in several cases and were 3 per cent or above in 4 instances. In vitro studies on heated blood showed no alterations with temperatures as high as 46°C. Above that point changes occurred.

In patients with severe or moderately severe thermal burns, the red cells examined promptly after the burns exhibited changes in morphology and osmotic fragility similar to those obtained by the injection into dogs of the animals' own erythrocytes heated in vitro to approximately 53°C. The increased osmotic fragility of heated red cells apparently results from conversion of the normal biconcave erythrocytes to more nearly spherical forms by a process of progressive fragmentation. The mechanism by which heat causes fragmentation of red cells with increased osmotic fragility in vitro occurred through a mechanism of swelling and osmotic hemolysis in the isotonic plasma of the animal.

Shen, Ham, and Fleming concluded: "In thermal burns a significant number of erythrocytes may be destroyed by heat, probably depending on the temperature attained by the blood, the duration of heating and the volume of blood subjected to these conditions." Netsky and Leiter (1943) noted in their experiments that "intravascular hemolysis and escape of hemoglobin into the lymphatics is a consequence of severe burn."

In general, it can be said that hemoglobinemia and associated hemoglobinuria

are often present during the first day or so after a very severe burn. Shen, Ham, and Fleming (1943) showed that such hemoglobinemia usually results from direct trauma to red cells in the capillaries of the burned skin, while the precipitation of hemoglobin in the kidneys may contribute to the oliguria that often is noted. Lund (1943) gave the following advice in this regard: Patients with severe burns "should be catheterized at once and hemoglobin sought in the urine. Also the first blood specimen taken for hematocrit determinations should be used to test for hemoglobinemia. If either hemoglobinemia or hemoglobinuria are present, the patient should be alkalinized with intravenous injections of 40 cc. of 1 M lactate to each 500 cc. of plasma or an equivalent amount of sodium bicarbonate. As soon as an alkaline urine is established, the sodium lactate administration should be stopped." Rosenthal (1943) and Fox (1944) also found alkaline solutions to be of benefit in burns as discussed under sodium therapy. Hemoglobinuria was also noted by Lucido (1940) and many others. Bingold (1944) of Nurnberg believes that a definite renal causative factor may exist in these cases. Co Tui, Wright, Mulholland, Barcham, and Breed (1944) pointed out that the protein in the hemoglobin may account for some of the early urinary nitrogen losses reported in the literature. Olson, Walker, and Necheles (1944) also noted a hemoglobinemia in dogs burned under nembutal anesthesia. In one of their animals, the plasma contained 1385 mgm. per cent of hemoglobin 4 hours after the burn and in another instance, 45 minutes after the burn the plasma contained 3680 mgm. per cent of hemoglobin. They concluded "The severity of the anuria in all burn experiments frequently could be associated with the degree of hemoglobinemia."

The work of Mendelsohn and Rossiter (1944) on the subcutaneous temperatures in moderate temperature burns may have a bearing on the production of hemoglobinemia from overheating of blood. Temperatures were measured with a copper-constantin thermocouple inserted through a hypodermic needle under a burned area of guinea pigs. One minute application of a burning iron at 70°C. caused subcutaneous temperatures to rise to an average of 54°C., sufficient to injure red blood cells. Harkins and Harmon (1937) also made observations on the insulating power of living skin.

Recent observations by Moyer, Coller, Iob, Vaughan, and Marty (1944) studied the early masked anemia in burns from the therapeutic standpoint. Anesthetized dogs were immersed so that two-thirds of the body surface was scalded at 85°C. for 30 seconds. They concluded that hemoconcentration per se is not harmful and "the hemoconcentration of severe thermal injury does not contraindicate the transfusion of whole blood." They found whole blood to be superior to serum and "the combination of massive transfusions of defibrinated blood and saline-bicarbonate solution by stomach was the only form of therapy employed that prevented shock without inducing complications that were incompatible with life."

Ham (1944) reported: "The dilatation and congestion of both the smaller and larger blood vessels associated with the various types of burns studied in these experiments, together with the small hemorrhages that were not infre-

quently observed, serves to emphasize that extensive burns would tend to abstract significant quantities of whole blood from active circulation in addition to the plasma that is lost by leaking away from the smaller vessels."

Secondary anemia is a frequent occurrence after severe burns, in many cases coming on after the fifth day. It may be due to three chief causes: a) reabsorption of fluid, b) primary destruction or increased fragility of red cells due to the burn (Shen, Ham and Fleming, 1943), and c) continued destruction and slow formation of red cells due to sepsis, bleeding from granulating surfaces, and malnutrition in the late stages. The treatment consists in blood transfusions, a high protein, high vitamin diet, iron, and, in some cases, liver.

20. *Burn hypoproteinemia.* Early hypoproteinemia is rare although the total amount of plasma protein in the vascular tree may be drastically reduced. Such early hypoproteinemia when it does occur is often associated with excessive intravenous treatment with non-colloid solutions. Late in the course of burns hypoproteinemia is common and when granulating surfaces are present it tends to complete a vicious circle. The hypoproteinemia persists as long as the granulating surfaces remain and the latter will not heal as long as the hypoproteinemia interferes with their nutrition.

Hirshfeld, Williams, Abbott, Heller, and Pilling (1944) pointed out that patients who are moderately or severely burned may excrete more nitrogen in the urine than can be administered orally as protein without forced feeding. Second degree burns may show a temporary nitrogen deficit, but since most patients can stand such deficits for a short time it is in the third degree burns that maintenance of a high protein intake is essential. After three weeks of excessive protein catabolism such patients are still not only excreting abnormally large quantities of nitrogen in the urine, but are also losing considerable protein in the exudate from the burned surface. Using a specially prepared dressing, these authors studied the nitrogen loss from the burned surface. Closely woven glass cloth covered by a thick layer of cellucotton, and this in turn by an impervious covering of vinylite plastic bandage, was used to collect the exudate. Complete nitrogen balance studies were done on 6 patients with burns and it was found that from 3 to 35 per cent of the total nitrogen output occurred from the burned surface. These authors concluded: "Nitrogen continues to escape in significant quantities in the burn exudate until epithelization has occurred. This is an additional reason for the early skin grafting of large third degree burns."

Co Tui (1945) determined the extent of protein loss in burn exudates. In most cases nitrogen-free slabs of cellulose sponge were used for collection of the exudate. Ten 24 hour collections were made in 7 burn patients, at times varying from the second to the 94th day after the burn. The protein loss varied from 9.4 to 56.7 grams per day. This is equivalent to 150 to 945 cc. of plasma per day and is thus seen to be a tremendous loss. In four instances the area of the burn was determined by polar planimetry and the protein loss expressed as milligrams lost per square centimeter of area. Such loss varied from 5.6 to 20.4 mgm. of protein per square centimeter of open burned surface. Co Tui concluded: "The nitrogen losses were, in some cases, of sufficient magnitude to jeopardize recovery."

Urinary loss of excessive amounts of protein has been noted by many authors. Lucido (1940) reported that his patient had a high urinary excretion of nitrogen for the first 25 days following the burn. Browne (1942) studied the nitrogen output in three cases of burns. On a regular diet, all showed an elevated urinary output to as high as 28 grams per day, gradually decreasing to normal on the 40th to the 52nd day. Taylor, Levenson, Davidson, Adams, and McDonald (1943) reported an excretion of as much as 45 grams of nitrogen (equivalent to 250 grams of protein) a day. Cope, Nathanson, Rourke and Wilson (1943) reported lower values for the urinary excretion of nitrogen in burns and attributed the negative nitrogen balance in these patients to a low intake. Deaver, Cronkite, and Phillips (1944) also reported an abnormal nitrogen metabolism in a burned patient. Taylor, Levenson, Davidson, Browder, and Lund (1943) in a later paper, reported one case of a patient with 45 per cent of the body surface involved by a third degree burn who excreted as much as 34 grams of nitrogen in the urine with an average of 28 grams daily the first week. These authors found that correction of such a difference by high protein feeding failed to bring the patient into true nitrogen balance because of incalculable losses from the burned surface. However, heroic intravenous and tube feeding of about 300 grams of protein a day would restore his true balance, although at the time of writing he was still one-third below his normal weight.

21. *Nutritional factors.* Co Tui, Wright, Mulholland, Barcham, and Breed (1944) discussed the problem of nutrition in burns. These authors pointed out the possibility that the early protein loss reported by many writers may be partly accounted for by hemoglobinuria. They found an apparent mathematical relationship between the extent of the surface burned and the amount of nitrogen required to maintain nutrition. They pointed out that skin grafting in these patients with protein deprivation is especially liable to lead to shock. Although not stated in their paper, it should be mentioned that such cases should be skin grafted directly on top of the granulations, because removal of the latter may precipitate shock. Co Tui and his associates also reported that with adequate protein nutrition the necessity for blood transfusions was reduced to a minimum. This is in agreement with observations of Evans (1944).

Elman, Charnas, and Davey (1943) used an enzymic hydrolysate of casein containing a mixture of amino acids and polypeptides in the treatment of protein-depleted dogs and found that 1.75 grams of nitrogen per kilogram per day produced a positive nitrogen balance. This aspect of burn treatment was discussed in a recent paper by Aguilar (1944).

Taylor (1944) and Taylor, Davidson, and Levenson (1944) discussed the problem of protein nutrition in serious thermal burns. In the latter paper they stated: "It is a safe minimum margin that the severely burned patient will require as a minimum 2 to 3 grams of protein and at least 60 calories per kilogram of body weight *from the earliest possible moment* if weight loss and protein deficiency are to be *prevented.*" However, when protein deficiency is already present, more urgent therapy is required, "as 500 grams of protein by intubation and by vein may be needed per day to accomplish this result in a reasonable time. *It seems obvious, therefore, that the successful treatment of patients with a*

potential nitrogen deficiency lies in an early appreciation of the nitrogen demand and the immediate institution of remedial measures to prevent its occurrence."

Clowes, Lund, and Levenson (1943), reporting on 150 cases of burns, 109 of whom were victims of the Cocoanut Grove disaster, epitomized the need for nutritional care of burns as follows: "All patients with 10 per cent of surface area or more involved in third-degree burns became serious nutritional problems because of the loss of nitrogen in the urine and from the surface, and because of the increased nutritional requirements resulting from infection with fever."

22. *Metabolic factors.* Cope, Nathanson, Rourke, and Wilson (1943) pointed out that a disturbance in nitrogen balance is one of the most notable features after thermal burns. This subject has already been discussed in the last two sections. A decreased metabolic rate with resultant anoxic changes in the presence of severe shock is another important metabolic alteration. The reviews of McClure (1939) and of Lam (1941) are pertinent to this aspect of the subject.

Recent observations of Harkins, Long, and associates (1945) concerning metabolic changes are as follows: In all instances burns (scalds) were produced by immersing white male rats of standard size in hot water of constant temperature for a definite period of time. From such an injury there resulted a rise in plasma amino nitrogen, a slight rise in plasma ammonia nitrogen, a slight rise in liver amino nitrogen, practically no change in liver ammonia nitrogen or liver amide nitrogen, and no early decrease in oxygen uptake of liver slices but a late slight decrease, as measured by the Warburg apparatus. The significance of these findings is best explained by correlating them with the observations of Engel, Winton, and Long (1943) on hemorrhagic shock. It is enough to say, however, that many of these changes appear to be of metabolic origin rather than directly produced by the burned area.

Cope, Nathanson, Rourke, and Wilson (1943) found in their burn cases that the 17-ketosteroid excretion was elevated during the period corresponding to the negative nitrogen balance. This observation brings the subject of metabolic disturbances in burns to a consideration of a possible relationship to endocrine factors, as discussed in the next section. The Boston group reported an abnormal growth of hair in six women patients hospitalized for more than three weeks, despite low 17-ketosteroid levels.

23. *Endocrine factors.* Early observations indicating that adrenal damage is common in burns are reviewed by Harkins (1942). Cope and his associates (1943) noted the increased 17-ketosteroid excretion as well as an abnormal growth of hair in the six women hospitalized for more than six weeks, despite a low 17-ketosteroid level, referred to above. These and other observations have suggested a possible endocrine disturbance in burns. Recent work of Harkins, Long and associates (1945) has shown the presence in burns of not only a disturbance of one gland but an interrelationship between two glands. After the production of standardized scalds in anesthetized rats, there was a significant fall in adrenal total cholesterol (e.g., from a normal of 4.2 mgm. per 100 mgm. of adrenal weight to as low as 0.7 mgm.). This fall in adrenal total cholesterol did not occur in hypophysectomized rats, indicating that the effect of the burn may not be di-

rectly on the adrenal gland but is mediated by way of the pituitary, possibly by a stimulation of production or outpouring of adrenotropic hormone.

After burning there is a rise in blood sugar, both in man (Underhill, et al., 1923; Davidson, 1925; McIver, 1933; and Lambret, et al., 1936) d in experimental animals (Schreiner, 1926; Greenwald and Eliasberg, 1926; Lundberg, 1929; Beard and Blalock, 1931; Slocum and Lightbody, 1931; and Stolfi, 1936). Taylor, Levenson, and Adams (1944) reported observations on the abnormal carbohydrate metabolism in human thermal burns, emphasizing especially the occurrence of hyperglycemia. In a group of 35 consecutive burn patients, none of whom were known diabetics, admitted to the Boston City Hospital, 20 were in a hyperglycemic state on entry. "The initial Folin blood sugar level varied from 132 to 352 mgm. per 100 cc. . . . The occurrence of hyperglycemia was directly related to the extent of the burn. . . . The hyperglycemia persisted over a period of hours in all cases, and over a period of days in some. None of the patients studied showed a return of an elevated blood sugar level to normal within 12 hours." Associated with the hyperglycemia was an initial decrease in carbon dioxide combining power and a lactacidemia in certain cases. Thirteen of the 21 cases of hyperglycemia had associated hemoconcentration. While such blood sugar responses may be due to the pancreas, they may also be caused by hepatic involvement.

Clark and Rossiter (1944) working on rabbits and rats, also studied carbohydrate metabolism after scalds. Their observation can be summarized as follows: Burning caused a rise in the blood sugar of both rats and rabbits anesthetized with ether. In rabbits, this rise was greater in well-fed animals than in those starved for 24 hours, and had completely disappeared 24 hours after burning. In both species, adrenaline injections produced a similar hyperglycemia. In rats, whose adrenals had been removed, there was no rise in blood sugar after burning. Rats had a higher blood lactic acid one hour, but not 3 hours, after burning; adrenaline injections produced similar changes in blood lactic acid. Both 1 and 3 hours after burning, there was a fall in the glycogen content of the whole carcass (chiefly muscle glycogen) of rats. Similar changes were observed after adrenaline injections. The carcass glycogen of burned adrenalectomised animals remained normal. Rabbits, deprived of food for 24 hours and burned, had a lower liver glycogen, both 4 and 24 hours after burning, than the controls, which had been merely anesthetized. On the other hand, burning produced no change in the liver glycogen of either normal or adrenalectomized rats starved for 24 hours, but the injection of adrenaline caused a slight rise. Liver slices from rabbits killed 4 hours after burning formed glycogen from glucose less readily than slices from anesthetized control animals. Slices from similar animals, killed 2 to 4 hours after the injection of adrenaline which produced a similar degree of hyperglycemia, formed glycogen normally. Liver brei from burned rabbits did not break down glycogen, either by phosphorylase or amylase activity, any more rapidly than a similar brei from control animals. There was no increase in the amylase activity of serum of rabbits either 4 or 24 hours after burning. There was a fall in the ascorbic acid content of the adrenal gland of

rabbits, both 4 and 24 hours after burning, but no change in the ascorbic acid of the liver. Harkins (unpublished data) has found a similar fall in adrenal ascorbic acid after burning in rats.

Clark and Rossiter also found that injection of 5 ml. of isotonic ammonium chloride intraperitoneally into rats caused a rise in blood sugar of the same order as that observed after burning, but the plasma alkali reserve, after such an injection, was much less than that observed after burning. Intraperitoneal injection of 5 ml. of isotonic sodium bicarbonate did not influence the burn hyperglycemia any more than a similar injection of 5 ml. isotonic sodium chloride. They concluded that acidosis is thus not a major factor in the production of rat burn hyperglycemia. Well fed rats were no better able to survive burning than similar animals starved for 24 hours, leading to the assumption that a high blood sugar plays no striking rôle in the protection of rats from the ill-effects of burning. However, as a practical measure, it was suggested that glucose might be of benefit in the treatment of burns because of glycogen depletion that may result in such cases. The source of the blood glucose seemed undoubtedly to be chiefly muscle glycogen, and it was postulated that it is mobilized by a mechanism similar to the Cori cycle: muscle glycogen is mobilized in the form of lactic acid, which, in the liver, is transformed, possibly by way of glycogen, to glucose. Finally, these authors concluded that, as is true for other types of hyperglycemia, there are two mechanisms contributing to the high blood sugar which follows burning:

(1) The liberation of adrenalin from the adrenal glands.

(2) Some other process or processes, either stimulating hepatic glycogenolysis or inhibiting glycogenesis.

Some Unsolved Problems. Much recent progress has been made in elucidation of the problem of thermal burns due to the stimulus of the present war. Numerous important and practical questions based on the 23 items discussed in this paper still remain unanswered. A few of these are listed as a stimulus for future research:

1. Is a primary debridement of an early burn necrotic lesion advisable?
2. How can burn infection be controlled?
3. Should fresh burns always be cleansed?
4. Can the separation of burn sloughs be hastened or should that process be allowed to run its natural course?
5. The same question can be asked concerning the healing of burn wounds.
6. What are the relative advantages of plasma and of whole blood in the treatment of burn shock?
7. Is sodium therapy of specific value in the treatment of burn shock and/or of burn toxemia?
8. Does burn toxemia exist, and if it does, how can it be controlled?
9. How can burn anuria be controlled and is it toxic in origin?
10. What is the relation between the continuous stupor or delirium of some serious burns and the local lesion?
11. Is the treatment of burn anemia and hypoproteinemia a simple matter of adequate protein feeding or do the two conditions represent separate problems, each of a more complex nature?

12. Exactly what are the metabolic and endocrine changes in burns and do they offer any previously unconsidered leads to treatment?

Expressed succinctly, burn shock, burn toxemia, and burn sepsis still represent the three most important obstacles to the recovery of a burned patient.

SUMMARY

Some of the more important local alterations and processes in burns considered in this article include necrosis, edema, increased lymphatic flow, separation of burn sloughs, and healing and regeneration. General problems discussed include the use of morphine and anesthesia; burn shock, toxemia, anemia and hypoproteinemia; plasma and sodium therapy; the influence of environmental temperature on recovery, and certain metabolic and endocrine relationships in burns.

Burns form a prime example of a type of trauma with marked local and widespread general effects. In considering either the mechanism of any alteration associated with burns or the treatment of any phase of burns, both the local traumatic lesion and the general disturbances of the burned patient must be considered. Burn therapy should be a continuous process with correlation of the local, general, and skin grafting phases.

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